

**THIS WORK IS  
DEDICATED TO  
MY BELOVED FAMILY**

**PHYTOCHEMICAL INVESTIGATIONS ON SOME  
PLANTS OF BUNDELKHAND REGION**

**A THESIS SUBMITTED FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY IN CHEMISTRY TO THE  
BUNDELKHAND UNIVERSITY, JHANSI**

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# CONTENTS

	Page No.
CERTIFICATE	i
ACKNOWLEDGEMENTS	ii-iii
ABSTRACT	iv-viii
CHAPTER I	1-33
<div style="text-align: center;">INTRODUCTION</div> <p>Scope of phytochemical studies, modern methods of plant analysis, some important plant constituents and recent investigations made in their fields. Problem taken and work done and relevant bibliography.</p>	
CHAPTER II	<div>Isolation and study of a novel acylated flavonol glycoside; 6-[2''-hydroxy-3''-methyl butyl] quercetin -7-O-<math>\beta</math>-D-(2'''-galloyl)-<math>\beta</math>-D-glucopyranoside" from <i>Kickxia ramosissima</i> (wall.) Janchen syn. <i>Linaria ramosissima</i></div> <div style="text-align: right; vertical-align: bottom;">34-74</div>
CHAPTER III	<div>isolation and study of a novel flavone glycoside, "8-prenyl-chrysoeriol-4'-(<math>\alpha</math>)-<math>\beta</math>-D-xylopyranosyl-(1<math>\rightarrow</math>2)-<math>\alpha</math>-L-arabinopyranosyl-(1<math>\rightarrow</math>6)-<math>\beta</math>-D-galactopyranoside from <i>Kickxia ramosissima</i> (Wall.) janchen syn. <i>Linaria ramosissima</i></div> <div style="text-align: right; vertical-align: bottom;">75-107</div>
CHAPTER 4	<div>isolation and identification of a triterpenoid saponins stems of <i>Echinocystic acid</i>-3-O-<math>\beta</math>-D-galactopyranoside, from the stems of <i>Ziziphus nummularia</i> (fam. Rhamnaceae).</div> <div style="text-align: right; vertical-align: bottom;">108-144</div>
CHAPTER 5:	<div>isolation and identification of a triterpenoid, 3, 21-dihydroxy - olean - 12 - en - 28 - oic acid, from the aerial parts of <i>Celsia coromandeliana</i>"</div> <div style="text-align: right; vertical-align: bottom;">145-162</div>
APPENDIX	<div>copies of accepted and communicated Research papers.</div>

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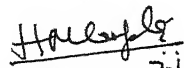
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## CERTIFICATE

This is hereby certified that the thesis entitled  
"Phytochemical Investigations on Some Plants of Bundelkhand Region"  
submitted by Mr. Neeraj Srivastava, embodies his own research work  
and he worked under my supervision over the minimum period required  
under para 7 of the Ph. D. ordinance and candidate has put the required  
attendance in the department during the research period.

In my opinion, the thesis fulfils the requirement of the  
ordinance relating to the Ph. D. degree of Bundelkhand University  
Jhansi (UP) India.

  
H.M. Gupta 7.11.2000

[Supervisor]

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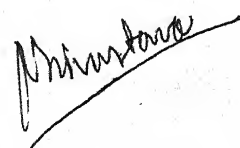
This will be the greatest pleasure for me to thank my friends, seniors & colleagues Dr. Ajay Dixit (CIMAP LKW.), Mr. Sanjeev Tripathi, Dr. Shailendra Badal (Alld. Uni.), Mr. Ashok S. Parihar, Dr. (Mrs.) Renu Bhatt, Dr. (Mrs.) Varsha Kanchan, Dr. (Mrs.) Pragya Khare, Miss Anita Gupta, Dr. Kishor Srivastava, Dr. M.A. Ansari and Dr. N.C. Agrawal (Sagar Uni.).

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With all the blessings of Bhagwan Mahakaleshwar Ujjain, I am submitting this work.

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## ABSTRACT

This thesis entitled 'Phytochemical Investigations on some plants of Bundelkhand region' consists of five chapters and deals with the phytochemical studies made on the (i) aerial parts of *Kickxia ramosissima* (Wall.) Janchen Syn. *Linaria ramosissima*, (ii) stems of *Ziziphus nummularia* and (iii) aerial parts of *Celsia corromandeliana* (Vahl.). A brief description of the five chapters is given below:

### CHAPTER 1:

#### INTRODUCTION:

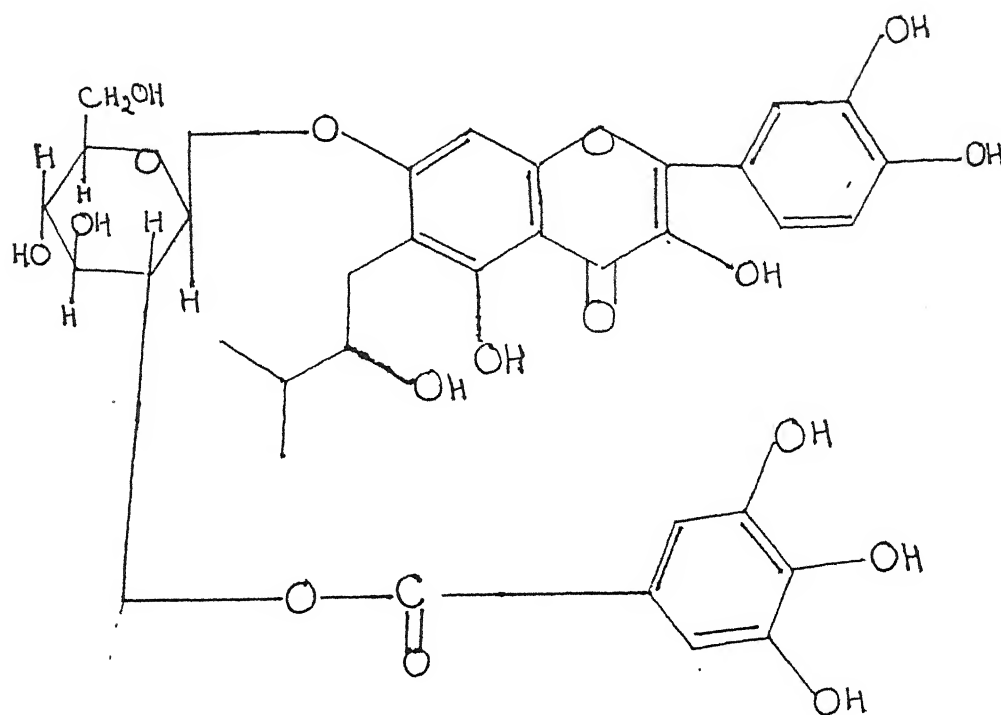
The chapter 1 is an introductory one and deals briefly with the historical facts, relevance, scope and the future prospects of the plant chemistry. It also gives in short the applications of modern methods of isolation, separation and the structural determination viz. chromatographic and spectroscopic techniques. This chapter also covers the brief account of some of the important allelochemicals along with the important features of the problem taken and the actual work done with relevant bibliography.

### CHAPTER 2:

ISOLATION AND STUDY OF A NOVEL ACYLATED FLAVONOL GLYCOSIDE; 6-[2''-HYDROXY-3''-METHYL BUTYL] QUERCETIN -7-O- (2'''-GALLOYL)- $\beta$ -D-GLUCOPYRANOSIDE" FROM *KICKXIA RAMOSISSIMA* (WALL.) JANCHEN SYN. *LINARIA RAMOSISSIMA* (FAMILY- SCROPHULARIACEAE).

A novel flavonoid glycoside was isolated from the  $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$  (4:6) fraction of ethyl acetate soluble part of 95% methanolic extract of aerial parts of *Kickxia ramosissima* (Wall.) Janchen syn. *Linaria ramosissima*. The compound was analysed for the molecular formula  $\text{C}_{33}\text{H}_{34}\text{O}_{17}$ , m.p.  $256-257^\circ$ ,  $M^+$  702. This

compound was identified as 6-[2''-hydroxy-3''-methyl butyl] quercetin -7-O- (2'''-galloyl)- $\beta$ -D-glucopyranoside (I) on the basis of colour reactions, chemical degradation and UV, IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and Mass spectroscopy.

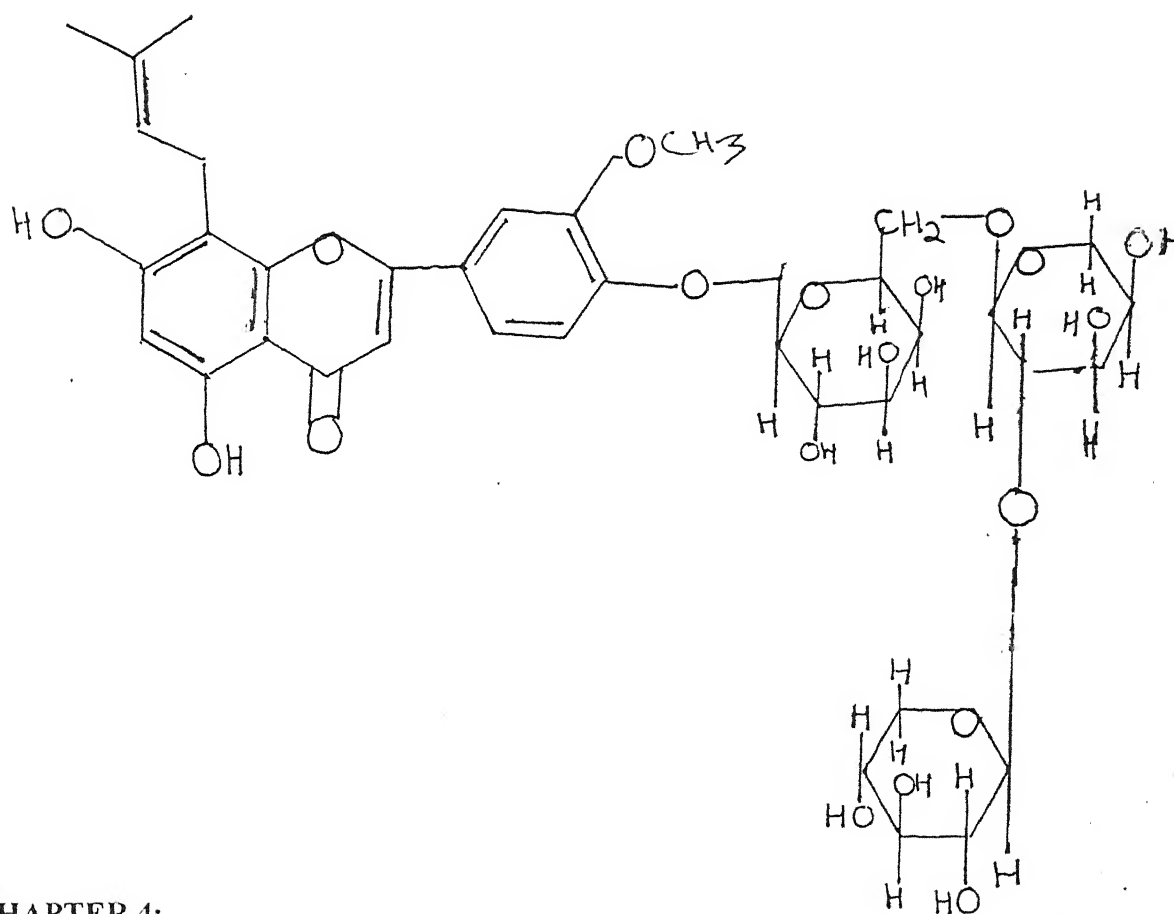


### CHAPTER 3:

ISOLATION AND STUDY OF A NOVEL FLAVONE GLYCOSIDE, "8-PRENYL-CHRYSOERIO-4'-O- $\beta$ -D-XYLOPYRANOSYL-(1 $\rightarrow$ 2)- $\alpha$ -L-ARABINOPYRANOSYL-(1 $\rightarrow$ 6)- $\beta$ -D-GALACTOPYRANOSIDE" FROM *KICKXIA RAMOSISSIMA* (WALL.) JANCHEN SYN. *LINARIA RAMOSISSIMA* (FAMILY -SCROPHULARIACEAE).

This chapter of the thesis incorporates the structural determination of a novel flavonoid glycoside molecular formula  $\text{C}_{37}\text{H}_{46}\text{O}_{19}$ , m.p. 308-309°,

molecular weight 794 (EIMS). This was isolated from the  $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$  (1:9) fractions of the extract, obtained from the chapter 2. The flavonoid glycoside II was characterised as “8-prenyl-chrysoeriol-4'-O- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-galactopyranoside” (II) on the basis of colour reactions, chemical analysis, UV, IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and Mass spectroscopy.



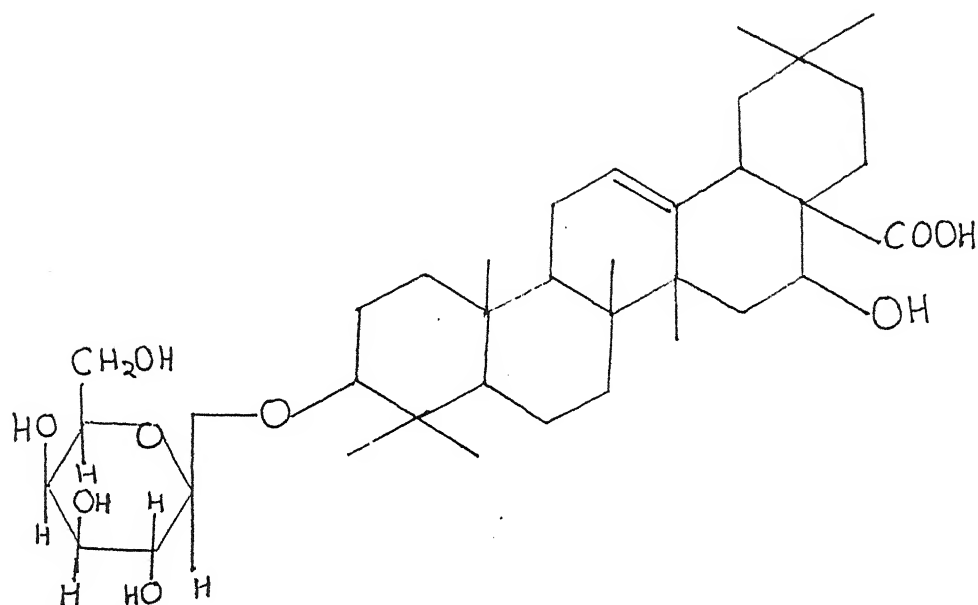
#### CHAPTER 4:

ISOLATION AND IDENTIFICATION OF A TRITERPENOIDAL SAPONIN GLYCOSIDE: “ECHINOCYSTIC ACID -3-O- $\beta$ -D-GALACTOPYRANOSIDE” FROM THE STEMS OF *ZIZIPHUS NUMMULARIA* (FAM. RHAMNACEAE).

This chapter of thesis includes the structural elucidation of a triterpenoidal saponin ) analysed for molecular formula,  $\text{C}_{36}\text{H}_{58}\text{O}_9$ , m. p. 251-

253° and  $M^+$  634 (EIMS) and was extracted from the 80% ethanolic extract of stems of *Ziziphus nummularia*.

The structure of compound was ascertained as echinocystic acid -3-O- $\beta$ -D-galactopyranoside on the basis of colour reactions, chemical analysis, UV, IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and Mass spectroscopy.



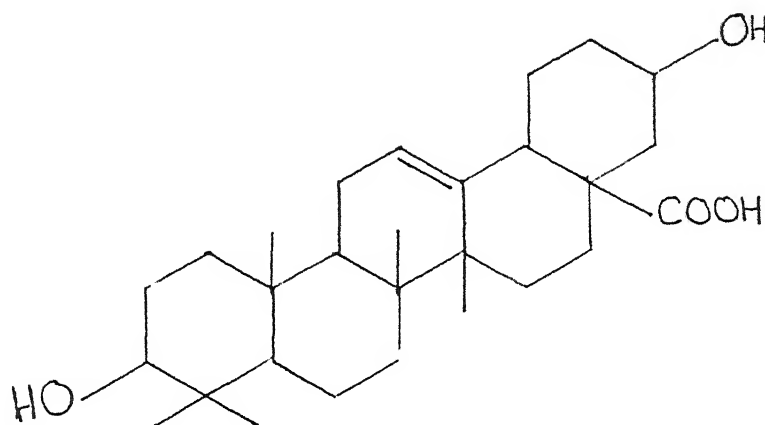
## CHAPTER 5:

ISOLATION AND IDENTIFICATION OF A TRITERPENOID, "3, 21-DIHYDROXY - OLEAN - 12 - EN - 28 - OIC ACID" FROM THE AERIAL PARTS OF *CELSIA COROMANDELIANA*"

The methanolic extract of the aerial parts of *C. coromandeliana* (Vahl.) yielded a triterpenoid, 3, 21-dihydroxy - olean - 12 - en - 28 - oic acid,

molecular formula  $C_{30}H_{48}O_4$ , m.p. 292-293° and  $M^+$  472 (EIMS). It was soluble in methanol and ethanol and responded positively to all the tests of triterpenoids.

In this chapter, the structure of this compound is ascertained by the chemical and spectral techniques viz. IR,  $^1H$  NMR,  $^{13}C$  NMR and Mass spectroscopy.



## CHAPTER I

### INTRODUCTION

## INTRODUCTION

Disease, decay, inabilities and death have always coexisted with the life and have made otherwise wonderful life of the man plaguing. These adversaries have even threatened the very existence of the life of the human race. It is therefore obvious for him to find the ways and means to ward off the disease. In his search of the substance with curative value man has made a vast progress. During this century chemical scientists have synthesised various potent drugs viz. antibiotics, antivirals, antimalarials, and sulphonamides which are useful in the treatment of various bacterial, viral and protozoa diseases and served greatly to the mankind.

Nevertheless, there is another side of the coin also that with the growth of jungle of industries for producing these drugs at the cost of balanced ecosystem; the man has made himself foe of environment. But the nature has its own ways to retaliate against this tempering with the balance of ecosystem, which has forced us to evolve ecofriendly system in every field and derive new means to bring back harmony with the nature which at this stage, is quite a hard task. Our own discovery of new drugs have been found, in some cases, acting as pollutants of nature, and in several cases have shown immediately or experienced later in the life or much later through effects on the coming generations. Thus, the use of synthetic chemicals in every field is being discouraged. Plants are unique chemists, they synthesise variety of chemicals during their routine life reactions in order to protect themselves from the herbivores viz. insects, grazers and against pathogens. These chemicals can be subdivided into two categories as primary metabolites viz. proteins, carbohydrates, vitamins, etc. and secondary metabolites which are regarded as "allelochemicals". Many a times these allelochemicals directly or indirectly affect human /animal health

on their ingestion either due to their therapeutic value or toxic nature. Most of the plants, which are harmful to man in large quantities, provide good medicinal products for various diseases on their use in small, and regulated quantities.

While man has been present on this planet for nine lakh years, modern medicine is just fifty years old. Had this system been essential to preserve the population from death, we would have got extinct generations ago. The fact is that the human race has survived not because of drugs but because of its rich knowledge of herbal medicines, gained by the experience of generations. For centuries, our ancestors have been using different plants and herbs for the treatment of different ailments. During the ancient times Aryans in our country used herbs and plants as medicines and the description of these plants is mentioned in the holy Vedas written between 4500 and 1600 BC Susruta Samhita and Charak Samhita also contain detailed information on the drugs derived from the plants.

Not only Aryans in our country but Chinese and Egyptians were using plants for curing various physical complications on other parts of the globe too. For example, in eastern Mediterranean countries the local physicians often prescribe the decoction of dried seeds of a local plant *Ammi visnaga* as a diuretic and as an antispasmodic in renal colic. Investigations by G.V. Andrep<sup>1</sup> and his colleagues in Cairo showed the active constituents to be Khellin, which, was found to be an effective vasodilator with a selective action on the coronary arteries. Subsequent clinical trials have demonstrated the Khellin's use in the treatment of Angina pectoris. From the ancient times, the root of an indigenous plant *Rauwolfia serpentina*, has been widely used in India and Malaya as an antidote to insects and snake bites, as a febrifuge, as a stimulant to uterine contractions and as a sedative. R.J. Vakil<sup>2</sup>

investigated its use in hypertension and found it to have a remarkable hypertensive action.

The knowledge of ancient man was based on the experiences of generations, often of centuries and this is up to the present day researchers to utilise and exploit this vast knowledge in the benefit of present and coming generations on this wonderful planet. Isolating the beneficial substances from the plant sources will be more useful than to synthesise these chemicals because synthetic processes are tedious, expensive, hazardous and at the same time disturbing to the balance of ecosystem. The synthetic processes for which a chemist employs heat and pressure in a laboratory are effected in plants at ordinary temperature and pressure through its routine life reactions. For example, it became possible to synthesise quinine alkaloid after nearly 50 years of extensive work, whereas, the Cinchona plant does it without difficulty everyday.

These secondary metabolites also work as a chemical weapons to the plants against their enemies. The most conspicuous event in the history of angiosperms is the failure of insects and other herbivores to attack the plants on wide scale. It follows that all the plants must be broadly repellent to animals, as food and toxic in widest sense. It is the selective ability of insects and grazers to overcome these defence mechanisms that allows them the limited feeding that we witness today. These plants produce some toxic substances in their regular metabolism. These toxins often have the role of feeding repellents, since plants advertise their presence by a warning signal of visual or olfactory nature. Thus, animal may be made aware of the presence of toxins even before it starts feeding. According to the theory of biochemical coevolution, it should be possible to observe an evolving pattern of

feeding deterrents within the plant kingdom. As the angiosperms evolved, they should appear to have developed different modes of protection from animal feeding. This is in fact, apparent if one considers the various plant deterrents and their distribution in different plant families. Some deterrents are highly sophisticated in their action; they affect hormonal balance within the animal. Other deterrents are highly toxic (e.g. cyanogen alkaloids) and deter largely on account of their poisonous nature. Yet, other deterrents reduce palatability (e.g. cucurbitacins, tannins). Tree leaves and shrubs form major bulk of animal feed during the time of scarcity. The leaves are quite nutritious but often their nutrients are not fully utilised due to the presence of some detrimental allelochemicals<sup>3-6</sup>. In turn the use of such tree leaves and bushes as regular fodder results poor health performance, abnormal reproduction behaviour or some times instantaneous deaths.

These secondary metabolites or better termed as allelochemicals which are synthesised by the plants in their regular metabolism for combating feeding are mostly harmful to man in large quantities, but provide good medicinal products for curing various diseases on their consumption in small and regulated doses. Our knowledge is rich and advanced so far as the human race is concerned but poor in veterinary sciences. The distribution as described in literature for medicinal and poisonous plants is coarse.<sup>7</sup>

A good number of plants have been adopted in pharmacopoeia after systematic chemical and pharmacological investigations. However, far and large numerous are still waiting for inclusion to invigorate the indigenous system of medicine. In a country like ours, this type of research has a vast scope due to her diversified natural wealth specifically medicinal flora. A large number of plants have

found place in the literature through the vast experience of saints, sages, and scholars and are reported in the ancient medical literatures <sup>8-14</sup>. Majority of plants is still waiting for the investigations for assessment of claims made for them. Now it is up to the phytochemical researchers to explore this infinite treasure. Due to the benefits with out side effects, even people from urban areas are also reverting to the traditional Ayurvedic system; this is a very encouraging sign for the phytochemical research.

India is facing grave scarcity of medical facilities. As population is increasing unmanageably day by day, primary health care centres are becoming less available to a large proportion of population. As we are richly bestowed with diverse flora and fauna, this knowledge of medicinal plants can be used to cure the different ailments of people to serve the human race. This is research alone that can scientifically justify the benefits of these easily available herbal drugs, thus, enabling their use on a wide scale.

In this present era, we have made a vast progress to uncover the new techniques and technologies to facilitate the research on these herbal drugs. When a plant is chemically treated, some physical methods including chromatographic techniques are applied for the separation of its active constituents viz. paper<sup>15-19</sup>, column<sup>20-23</sup>, thin layer chromatography<sup>23-30</sup>, GLC and HPLC.

Among these chromatographic techniques GLC and HPLC are more advanced. Gas liquid chromatography or commonly called as GLC<sup>31-35</sup> is used for the separation of complex mixtures, works on the principle of different migrating speeds of compounds in a mixture when carried along by an inert gas. A recent advancement of GLC is the high performance liquid chromatography or popularly known as HPLC, in which separation is effected by the application of high pressure

and has been reviewed in the separation of many plant phenolics by different workers.<sup>36,37</sup> HPLC finds its use in the separation of those compounds, which are not easily handled by the GLC e.g. thermally labile, polymeric samples and highly polar compounds without prior derivatisation can be easily handled by the HPLC.

Besides these regular techniques there are some advanced techniques like Droplet Counter Current technique that was developed by Tarimura<sup>38</sup> for the characterisation of flavonoids. It possesses greater utility in the resolution of glycosidic mixture.<sup>39</sup>

Spectroscopic techniques have made the task of phytochemists very easy as these techniques have replaced the old classical methods which were time consuming, tedious and required lot of quantities of compound. These include UV, IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and mass spectra.

UV<sup>40-48</sup> spectroscopy has its importance in conjugated systems, particularly it has an edge in ascertaining the positions of OH, OMe and sugars in molecules with extended conjugation e.g. flavonoids by the use of various shift reagents.<sup>49,50</sup> In this region the electronic structure of the molecule determines the spectrum of the compound, in which on absorption of the energy, electron from the ground state orbital get promoted to the orbitals of the higher energy level. An UV spectra is a graphical representation between the wavelength or frequency of absorption versus the absorption intensity (absorbance or transmittance).

The electromagnetic radiations, which occur between visible and microwave region, are called infrared radiations. The useful portion for the organic chemist is between 4000 cm<sup>-1</sup> to 666 cm<sup>-1</sup>. This region presents a very complex spectrum even for simple compounds and it gives advantage to the chemists in

matching of an unknown compound with that of an authentic sample. Although it is an indication of entire molecule but certain groups give rise to bands at or similar to the frequency irrespective of the structure of rest of the molecule. There is a great amount of work available on I. R. spectrum that is published in no. of books,<sup>51-54</sup> and compilations.<sup>55,56</sup>

The proton magnetic spectrometry or better known as  $^1\text{H}$  NMR spectrometry has been proved to be of great value in determining the position, number and chemical environment of the proton of organic compounds, thus, facilitating the structural determination of unknown compounds.<sup>57-63</sup> The principle of this technique lies in the fact that nuclei of some elements behaves just like tiny bar magnets, when they are placed in magnetic field, they exhibit their presence and environment by the way of emitting radiations in the form of signals recorded by the spectrometer. This technique finds its use in fixing the positions of sugars, hydroxylation, and prenylation.<sup>64,65</sup>

In early seventies a new technique  $^{13}\text{C}$  NMR spectrometry<sup>66,67</sup> came into the notice of organic chemists to interpret the Carbon skeleton and C atoms in the Carbon containing functional groups. As  $^{13}\text{C}$  is magnetically active in contrast to  $^{12}\text{C}$ , when it is placed in magnetic field, it emits continuous radiations that are recorded.  $^{13}\text{C}$  resonance is expressed in terms of parts per million (PPM) relative to TMS and occurs over the range of 0-200 ppm. The position of signal relative to the TMS is a good sign to the type of C atom present in the compound. For example, the acetylation of a free phenolic hydroxy group produces a significant change in the  $^{13}\text{C}$  NMR spectrum of flavonoids<sup>68</sup> and is used to ascertain the position of OH groups. Although the  $^{13}\text{C}$  NMR data are helpful in establishing the oxygenation patterns of

flavonoids, they are most useful in defining sugars especially flavonoid C glycosides which are difficult to be identified by other methods.<sup>69</sup>

Mass spectrometry is primarily better than other techniques because it requires very minute quantity of sample for analysis. In a mass spectrum a series of signals are displayed representing different charged fragments, of the compound under analysis, produced as a result of electron impact within spectrometer. The signals are represented according to the molecular weight for each fragment or through molecular weight per unit charge of the fragment, thus, enabling the determination of precise molecular formulae for the molecular ion and all fragment ions. Therefore, ms not only help in determining the molecular weight but also the molecular formulae of the organic compound. There are various books and compilations available on this subject.<sup>70-74</sup> The fragmentation pattern of flavonoidal glycosides and methoxy and hydroxy flavonoids is characteristic and have been discussed by several workers. The loss of 15 indicates the presence of methyl group at 6 or 8 positions of flavonoids. A peak at  $M^+ - 17$  is a characteristic peak of isoflavones, flavonols and flavones with 2'-hydroxylation due to the loss of OH group. Prenylation of flavonoids adjacent to methoxy group is indicated by the appearance of  $M^+ - 56$  peak, whereas, loss of  $M^+ - 43$  is indication of prenylation adjacent to OH group.<sup>75</sup>

These modern techniques are employed to isolate the biologically active constituents from the plants and to ascertain their chemical structure that give them clinical value. These biologically active compounds or better termed as allelochemicals have always attracted phytochemists for the study of molecular structure, biological function, natural distribution, biosynthesis and economic value

viz. pharmaceutical, fungicidal, insecticidal, antibiotic and disinfectious etc. These allelochemicals fall in several categories viz. alkaloids, anthraquinones, flavonoids, isoflavonoids, saponins, steroids, and terpenoids etc. These plant constituents differ in quantity as well as quality according to the seasonal changes, difference in habitat, nature of soil and human interference during cultivation.

The flavonoid pigments are one of the important members of the constituents of the plant, imparting colour to the plants. They are phenolic in nature and have common basic structure of two phenyl rings linked by three carbon atoms ( $C_6-C_3-C_6$ ). Variation in the state of oxidation of central  $C_3$  fragment gives rise to many class of compounds like catechin, dehydrochalcone chalcone, flavone, flavonol, aurones, anthocyanins dihydroflavonols, isoflavonols, coumestans and pterocarpenoids etc. Their importance is due to their physical and biological activities and work on their chemistry, occurrence, natural distribution and biological functions continues to be unabated. Generally, these colouring matters occur as glycosides from which the true colouring matter termed as aglycone is obtained by hydrolysis. Glycosidic variation is very large but the occurrence and distribution of free flavonoids have been reviewed.<sup>76</sup> Distribution patterns of these compounds in Bryophyta, Pteridophyta, Gymnosperms and Angiosperms have been extensively reviewed from time to time.<sup>77-86</sup> Koike<sup>87</sup> established the relationship between the molecular structure of flavonoids and their biological activities and showed that there was increase of potency with the number of OH groups. The effect of flavonoids on hypertension, diabetes, rheumatic fever, arthritis, and pregnancy has been reported.<sup>88</sup>

Some of the flavonoids and other compounds of this type have been recently reported in different standard works.

- Sophoflavescenol a novel flavonoid from the roots of *Sophora flavescens*.<sup>89</sup>
- Three novel compounds, hydroxyalpinumisoflavone, ephedroidin and genisteone from *Genista ephecroides*.<sup>90</sup>
- Five novel compounds including a norlignan named virgatyne; a tannin, virganin ; and three flavonoid sulphonates, galangin-8-sulphonate, galangin-3-O- $\beta$ -D-glucoside-8-sulphonate and kaempferol-8-sulphonate.<sup>91</sup>
- Dioflorin, a minor flavonoid from the root bark of *Dioclea grandiflora*<sup>92</sup>
- A new flavone di-C-glucoside, violarvensin from *Viola arvensis*.<sup>93</sup>
- Four novel minor prenylated flavanones, abyssinone-V 4'-methyl ether and abyssinoflavanones IV, V, VI have been reported from *Erythrina abyssinica*, African medicinal plant.<sup>94</sup>
- A novel acylated flavonol glycoside gallate ester from *Acer okamotoanum*.<sup>95</sup>
- A novel flavone 4'-hydroxy-3,6-dimethoxy-6'', 6''-dimethyl chromeno (7,8,2'', 3'') flavone from *Citrus reticulata*.<sup>96</sup>
- Oven dried and grounded roots and flowers of *Clerodendron phlomidis* (fam. Verbenaceae) yielded a novel flavanone, flavonodiglycosides,  $\alpha$ -L-Rhamnopyranosyl (1  $\rightarrow$  2)-  $\alpha$ -D-glucopyranosyl-7-O-naringin 4'-( $\alpha$ -D-glucopyranoside-5-methyl ether and a chalcone glycoside, 4, 2', 4'-trihydroxy-6'-methoxychalcone-4, 4'- $\alpha$ -D-diglucoside<sup>97</sup>.
- The structure of 7-Desmethyartemein (5, 7-dihydroxy-3, 6, 3', 4'-tetramethoxyflavone); a rare flavonoid reported from *Limnophila gratissima* (Fam. Scrophulariaceae) was revised and was found to be a mixture of two

flavones [5,7-dihydroxy-6, 8, 4'-trimethoxy flavone (nevadensin) and 5-hydroxy-6, 7, 4'-trimethoxy flavone (salvigenin)<sup>98</sup>.

One of the isomeric forms of flavones is isoflavonoid Which is often referred as phytoestrogen<sup>99</sup> because it increases meat and milk production in animals and produces, "Infertility syndrome". After the recognition of Infertility syndrome in Australian sheep grazing on subterranean clover during 1924 by an isoflavonoid, genistein<sup>100</sup>, the chemistry of isoflavonoids got much importance. A number of estrogenic isoflavonoids have been isolated from clover, leuceum and other leguminous forages.<sup>101-104</sup> Although most of isoflavonoids were isolated from leguminosae, yet reports are available from other families too<sup>105,106</sup>.

Alkaloids are another prominent class of secondary metabolites and are distinguished from other natural compounds by having one or more basic N atoms. According to Ladenburg alkaloids are naturally occurring plant constituents (some times also found in animals) with strong physiological action, having basic character, and contain at least one N atom in the ring. Alkaloids occur frequently in flowering plants (at least one in 15 species)<sup>107</sup> These are very active in biological system. Number of alkaloids are used as poison and medicine viz. morphine, codein, papaverine and cocaine etc. The several novel alkaloids have been reported in different standard works<sup>108-120</sup>.

Another class of compounds is lactones, which are present in large number of organic compounds obtained from plants.<sup>121</sup> Steroidal lactones are mostly cardiac glycosides like digetoxigenin. Coumarins are also lactones. These also exhibit varied physiological activities<sup>122</sup> like photodynamism, haemorrhage, headache, etc. in animals particularly mammals.

Saponins are widely distributed class in plant kingdom and have been found to occur in more than 500 species belonging to more than 80 different genera of various families<sup>123</sup>. Saponins are the glycosides of polycyclic aglycone of either C<sub>27-29</sub> steroids or C<sub>30</sub> triterpenes and sugar. They form a molecular complex with cholesterol and other 3 $\beta$ -hydroxy steroids, which is utilised both for the isolation of saponins and for the separation and purification of 3 $\beta$ -hydroxy steroid digitonin. These are the substances giving soap like foam in aqueous solutions. They are highly toxic when injected intravenously and can produce haemolysis of red blood cells at high dilution. Their toxicity is due to their activity in lowering surface tension.

Saponins can be classified into two classes as triterpenoidal saponins and steroidal saponins.

Triterpenoidal saponins are widely distributed in nature having C<sub>30</sub> carbon system. They occur either as free triterpenes or combined with sugar forming saponins. They can be divided into three groups according to their basic structures:

- a. ambrein and squalene
- b. the tetracyclic triterpenoids
- c. the pentacyclic triterpenoids

First group consists of only two substances, ambrein which is tricyclic tertiary alcohol with two double bonds and squalene which is acyclic compound with six double bonds.

The second group is represented by lanosterol and euphol, includes methylated steroids.

Third group is subdivided into  $\alpha$ - amyrin group (ursolic acid group and  $\alpha$ -boswellic acid group),  $\beta$ -amyrin group (oleanolic acid and  $\beta$ - boswellic acid

group), and lupeol group (monohydric terpene group). Some recently reported<sup>124-145</sup> members of this group are as listed below :

- Two novel pentacyclic triterpenoids,  $1\alpha,2\alpha,3\beta,24$ -tetrahydroxyolean-12-en-28-oic acid and  $1\alpha,2\alpha,3\beta,24$ -tetrahydroxyursa-12,20(30)-dien-28-oic acid were isolated from the flowers of *Gentiana tibetica*.<sup>128</sup>
- A new saponin, boussinogside E 3-O-[[ $\beta$ -D-glucopyranosyl]-20(29)-ene-30-norhederagenin 28-O-( $\beta$ -D-glucopyranosyl) ester was isolated from the methanolic extracts of the tubers of *Boussingaultia baselloides*.<sup>134</sup>
- A set of friedelane triterpenoids were isolated from the stem bark of *Maytenus macrocarpa*.<sup>137</sup>
- Three new bidesmosidic triterpine saponins, zygophylosides I, L and M were isolated from *Zygophyllum gaetulum*.<sup>138</sup>
- A new saponin, Lathyrus saponin was isolated from *Lathyrus japonicus*.<sup>139</sup>
- Six novel oleanene glycosides were isolated from the aerial parts of *Spergularia ramosa* which possess gypsogenin or quillaic acid as aglycone.<sup>140</sup>
- Two major triterpenoidal saponins, saponariosides A and B from the whole plants of *Saponaria officinalis*.<sup>141</sup>
- Three novel cycloartane type triterpene glycosides, brachyosides A, B and C and one new glycoside, cyclocephaloside II were isolated from the roots of *Astragalus microcephalus*.<sup>143</sup>

Sterols are neutral and comparatively stable substances, which occur in free as well as in glycosidic forms and partly esterified with fatty acids. These are regular constituents of plant fats. When they are present as glycosides, they are called as steroidal saponins. Sugars are generally attached to 3- hydroxyl group in

them. These saponins mostly possess spiroketal side chain. Some of the important natural saponins are amolonin, digitonin, dioscorea, gitonin, kammonin, sapotoxin and sarasaponin. Several novel steroids and steroidal saponins have been recently described in various papers<sup>146-151</sup>.

- Steroidal Prosapogenins from *Dioscorea offersiana*<sup>147</sup>.
- Lycopersides A-C, three Stereoisomeric 23- Acetoxyspirosolan-3 $\beta$ -Ol  $\beta$ -Lycotetraosides from *Lycopersicon esculentum*.<sup>148</sup>
- Two plant Ecdysteroids, 22-epi-20-hydroxyecdysone and gerardiasterone have been recently isolated from *Serratula tinctoria* L. (Compositae)<sup>149</sup>.
- A new Bidesmosidic spirostanol saponin named aculeosides B was isolated from the underground parts of *Ruscus aculeatus*<sup>150</sup>.
- A new lanostanoid and six known ergosteroids were isolated from the fruiting bodies of *Ganoderma applanatum* and *Ganoderma neo japonicum*. The structure of new lanosteroid was characterized as 24- $\zeta$ -methyl-5 $\alpha$ -lanosta-25-one<sup>152</sup>.

Some of the other important class of plant constituents are irridoids, proteins, carbohydrates, essential oils, fixed oils, tannins, gum and resins etc.

## PROBLEM TAKEN AND WORK DONE

India is bestowed with the richest medicinal flora of the world and our forefathers, from the very beginning of the civilisation, knew the importance of this natural wealth as medicines. They had learnt from their experiences, often of centuries, to utilise this natural wealth to ward off their different sufferings. This knowledge is quite rich and has been compiled to develop the modern pharmacopoeia. After systematic chemical and pharmacological investigations, a very large number of plants have been adopted in the pharmacopoeia, however, there is still numerous are waiting to be exploited by us. Phytochemistry, alone can scientifically justify the benefits of these easily available herbal drugs, thus, enabling their use on a wider scale. The relevance of plant chemistry increases, as the synthetic process which we carry out in our laboratories, are tedious, hazardous, expensive, time consuming and at the same time are disturbing the balance of ecosystem of this planet. Thus, to get the ecosystem in order, use of synthetic chemicals in every field is being discouraged.

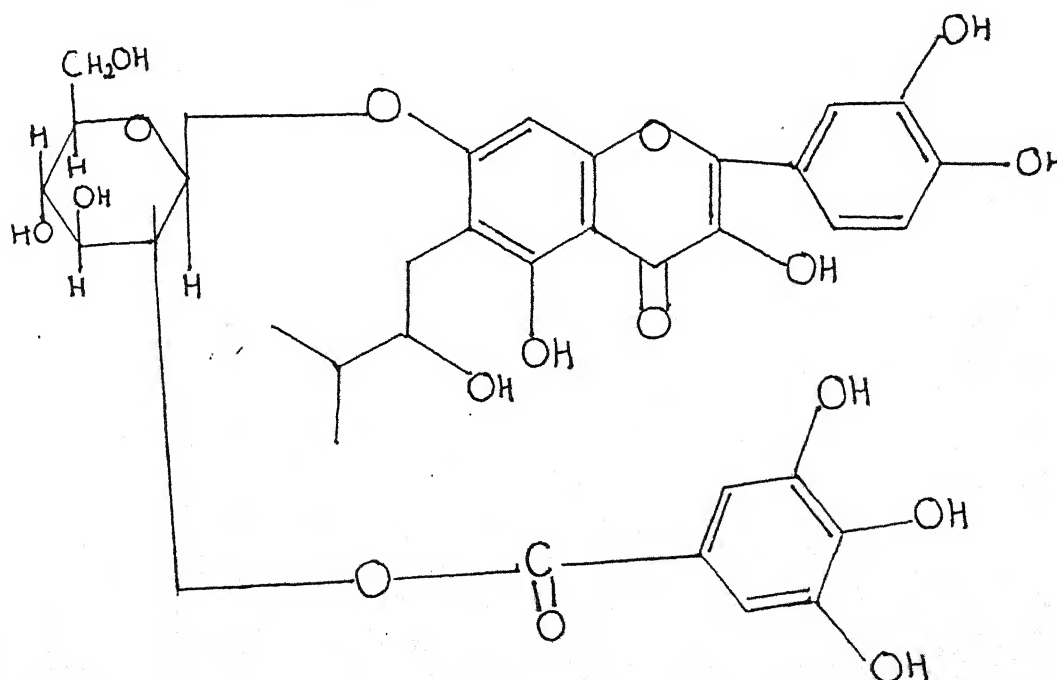
For the last fifty years, some serious studies have been going on in the field of phytochemistry resulting in the identification of a long list of the plants, which are associated with the medicinal properties but still there are numerous waiting for their inclusion in this list. Although these plants are associated with the phenomenal medicinal properties, yet they are not completely studied phytochemically.

The author was greatly influenced by the fascinating medicinal values of the following three plants available in this region a.) *Celsia coromandeliana* (Vahl.) b.) *Kickxia ramosissima* (Wall.) Janchen Syn. *Linaria ramosissima* c.) *Ziziphus nummularia* and therefore, decided that it is worthwhile to carry out investigations on them to isolate their active constituents and elucidate their

structures, so that, their therapeutic values can be correlated with these active principles. The outcome of the research is summarised below:

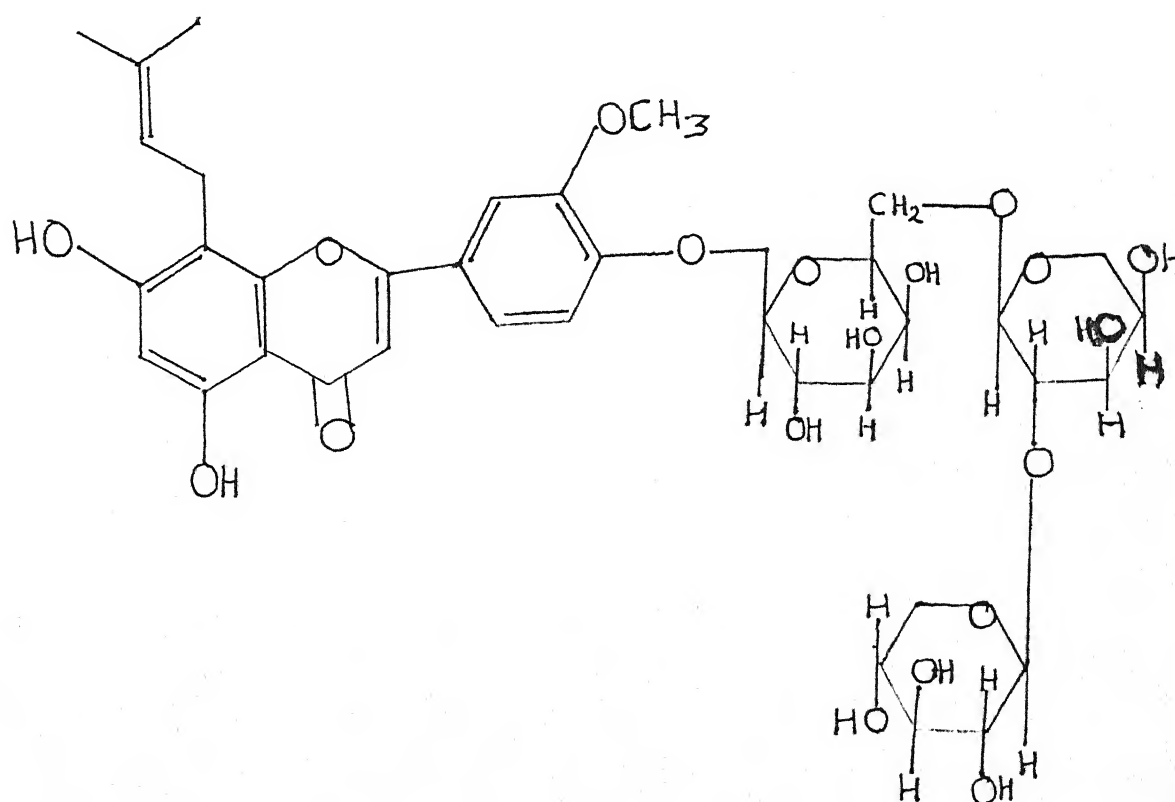
1.) ISOLATION AND STUDY OF A NOVEL ACYLATED FLAVONOL GLYCOSIDE; 6-[2''-HYDROXY-3''-METHYL BUTYL] QUERCETIN -7-O- (2'''-GALLOYL)- $\beta$ -D-GLUCOPYRANOSIDE'' FROM *KICKXIA RAMOSISSIMA* (WALL.) JANCHEN SYN. *LINARIA RAMOSISSIMA* (FAMILY-SCROPHULARIACEAE).

A novel flavonoid glycoside was isolated from the  $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$  (4:6) fraction of ethyl acetate soluble part of 95% methanolic extract of aerial parts of *Kickxia ramosissima* (Wall.) Janchen syn. *Linaria ramosissima*. The compound was analysed for the molecular formula  $\text{C}_{33}\text{H}_{34}\text{O}_{17}$ , m.p.  $256-257^\circ$ ,  $\text{M}^+$  702. This compound was identified as 6-[2''-hydroxy-3''-methyl butyl] quercetin -7-O- (2'''-galloyl)- $\beta$ -D-glucopyranoside (I) on the basis of colour reactions, chemical degradation and UV, IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and Mass spectroscopy.



2.) ISOLATION AND STUDY OF A NOVEL FLAVONE GLYCOSIDE, "8-PRENYL-CHRYSOERIO-4'-O- $\beta$ -D-XYLOPYRANOSYL-(1 $\rightarrow$ 2)- $\alpha$ -L-ARABINOPYRANOSYL-(1 $\rightarrow$ 6)- $\beta$ -D-GALACTOPYRANOSIDE" FROM *KICKXIA RAMOSISSIMA* (WALL.) JANCHEN SYN. *LINARIA RAMOSISSIMA* (FAMILY – SCROPHULARIACEAE).

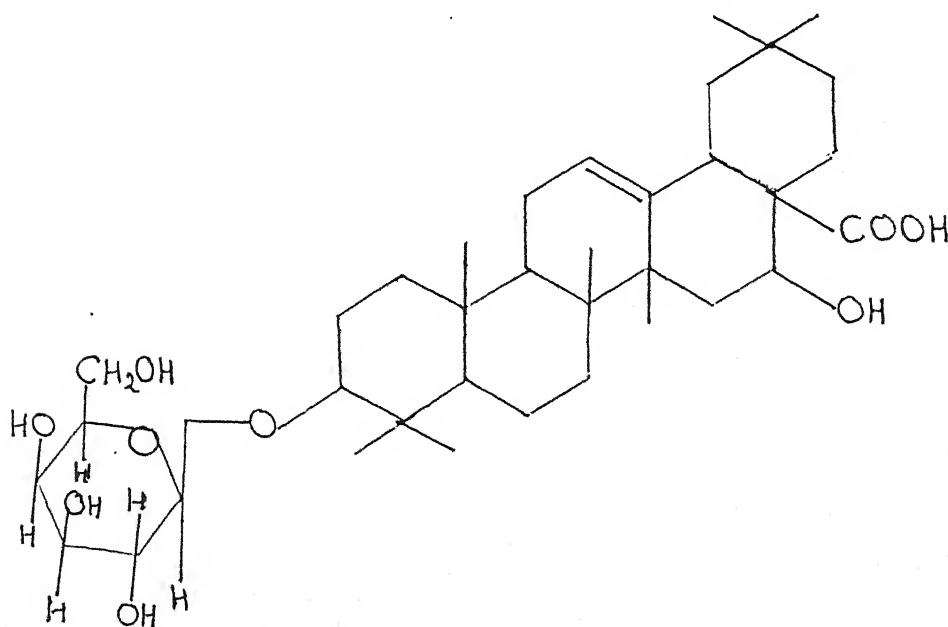
This chapter of the thesis incorporates the structural determination of a novel flavonoid glycoside molecular formula  $C_{37}H_{46}O_{19}$ , m.p. 308-309°, molecular weight 794 (EIMS). This was isolated from the  $CHCl_3$ :  $CH_3OH$  (1:9) fractions of the extract, obtained from the chapter 2. The flavonoid glycoside II was characterised as "8-prenyl-chrysoeriol-4'-O- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-galactopyranoside" (II) on the basis of colour reactions, chemical analysis, UV, IR,  $^1H$  NMR,  $^{13}C$  NMR and Mass spectroscopy.



3.) ISOLATION AND IDENTIFICATION OF A TRITERPENOIDAL SAPONIN  
GLYCOSIDE: "ECHINOCYSTIC ACID -3-O- $\beta$ -D-GALACTOPYRANOSIDE"  
FROM THE STEMS OF *ZIZIPHUS NUMMULARIA* (FAM. RHAMNACEAE).

This chapter of thesis includes the structural elucidation of a triterpenoidal saponin, analysed for molecular formula,  $C_{36}H_{58}O_9$ , m.p 251-253°. and  $M^+$  634 (EIMS) and was extracted from the 80% ethanolic extract of stems of *Ziziphus nummularia*.

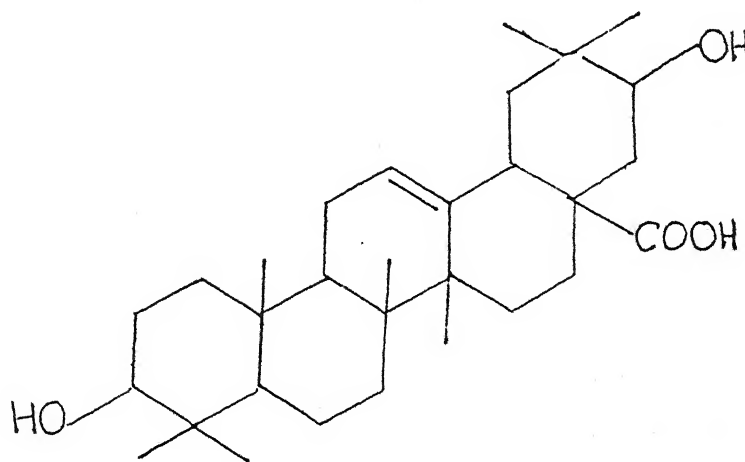
The structure of compound was ascertained as echinocystic acid -3-O- $\beta$ -D-galactopyranoside on the basis of colour reactions, chemical analysis, UV, IR,  $^1H$  NMR,  $^{13}C$  NMR and Mass spectroscopy.



4.) ISOLATION AND IDENTIFICATION OF A TRITERPENOID, "3, 21-DIHYDROXY - OLEAN - 12 - EN - 28 - OIC ACID" FROM THE AERIAL PARTS OF *CELSIA COROMANDELIANA*"

The methanolic extract of the aerial parts of *C. coromandeliana* (Vahl.) yielded a triterpenoid, 3, 21-dihydroxy - olean - 12 - en - 28 - oic acid, molecular formula  $C_{30}H_{48}O_4$ , m.p.  $292-293^\circ$  and  $M^+$  472 (EIMS). It was soluble in methanol and ethanol and responded positively to all the tests of triterpenoids.

In this chapter, the structure of this compound is ascertained by the chemical and spectral techniques viz. IR,  $^1H$  NMR,  $^{13}C$  NMR and Mass spectroscopy.



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## CHAPTER II

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ISOLATION AND STUDY OF A NOVEL ACYLATED FLAVONOL  
GLYCOSIDE; 6-[2''-HYDROXY-3''-METHYL BUTYL] QUERCETIN -7-O- (2''-  
GALLOYL)- $\beta$ -D-GLUCOPYRANOSIDE" FROM *KICKXIA RAMOSISSIMA*  
(WALL.) JANCHEN SYN. *LINARIA RAMOSISSIMA*

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*Kickxia ramosissima* (wall.) Janchen syn. *Linaria ramosissima* belongs to family Scrophulariaceae, the plants of which are found abundantly in ranges, grazing lands and they are cultivated too. The plants of family Scrophulariaceae, which includes 220 genera and 3000 species, are rich in various secondary metabolites often referred as allelochemicals viz. flavonoids<sup>1</sup>, isoflavonoids, saponins and sterols etc. These allelochemicals affect human/animal health when ingested either due to therapeutic value or due to toxic nature. Various genera of this family<sup>2-9</sup> have been examined extensively for the presence of flavonoidal constituents such as *Celsia coromandeliana*, *Grateola rateola*, *G. officinalis*, *G. linifolia*, *Kickxia aegyptiaca*, *K. lanigera*, *Limnophila gratissima*, *Linaria arvensis*, *L. dalmatica*, *L. genistifolia*, *L. japonica*, *L. pelisseriana*, *L. purpurea*, *L. simplex*, *L. transliensis*, *L. ventricosa*, *L. vulgariformis*, *L. vulgaris*, *L. kurdica*, *Striga aspera*, *Sopubia delphinifolia*, *Veronica agretis*, *V. opaca*, *V. ceratocarpa*, *V. persica*, *V. filiformes*, *V. autria* and *V. hederacifolia*.

*Kickxia ramosissima*<sup>10</sup> occurs throughout India usually on rocky and stony places and hanging downwards from the cervices and fissures of walls of old buildings.

The plant is a slender, glabrous, highly branched, perennial herb with upper alternate, lower opposite, petiolate, variable, round, ovate or hastate leaves along with solitary and axillary yellow flowers<sup>11</sup>.

The plant is vernacularly known as *Bhintgalodi* or *Kanodi* and is useful as a treatment for hyperglycaemia<sup>12</sup>.

The various genera of *Kickxia* have been investigated for their various constituents. Alkaloid peganine (vascine) was found in *L. dalmatica*, *L. genistifolia*, *L.*

*purpurea* and *L. ventricosa*. (1.) — peganine was isolated from *L. dalmatica*<sup>13</sup>. Oxodeoxypeganine, oxopeganine and an alkaloid were obtained from *Kickxia transliensis*, gathered in the stage of full blooming<sup>14</sup>. Flavonoids have also been reported from this genus. The whole plant of *K. lanigera* was found to be source of two flavones, 5,6,7- trimethoxy flavone and 5,6,7,4'-tetramethoxy flavone<sup>15</sup>. *Linaria kurdica* was investigated for the presence of acetyl pectolinarin, pectolinarigenin, linaroside and acacetin derivatives<sup>16</sup>. *K. aegyptiaca* afforded four flavonoids namely pectolinarigenin, its 7-O-robinoside and 7-O-rutinoside along with 4'''- acetate derivative of pectolinarigenin-7-O-rutinoside<sup>17</sup>. Three irridolinarins A, B and C; irridoid esters of irridoid glycoside were isolated from *L. japonica*<sup>18</sup>. Six *Linaria* sps have been examined for the presence of eleven irridoids. Eight out of these eleven irridoids were novel compound<sup>19</sup>. In the year 1995 four Bulgarian scientists<sup>20</sup> reported the irridoid composition of three *Kickxia* sps namely *K. elatina*, *K. spuria* and *K. commuta*. This study resulted in the isolation of two novel irridoid glycosides named as 5-O-menthiafoloyl kickxioside and its dimer kickxin as main components.

Therefore, in hope of the exploration of some compounds, which can define its medicinal properties, the author decided to undertake the detailed and systematic phytochemical study of *Kickxia ramosissima*.

#### ISOLATION OF THE FLAVONOID GLYCOSIDE:

Air-dried and finely powdered aerial parts (3.0 kg.) of *Kickxia ramosissima* were soxhlet extracted with 95% methanol. The extract was concentrated under reduced pressure to get a dark brown viscous mass, which was dissolved in cold water. The solution in water was partitioned with n hexane, benzene, chloroform and

ethyl acetate. The n hexane, benzene and chloroform extracts were very small in quantity, thus, they could not be studied.

#### STUDY OF ETHYL ACETATE SOLUBLE FRACTION:

The ethyl acetate soluble part was concentrated under reduced pressure to obtain a dark brown viscous material and subjected to column chromatography over a Si gel column. The column was eluted with  $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$  with increasing polarity. The  $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$  (4:6) part gave a homogeneous compound, which, on crystallisation with methanol yielded a yellow coloured micro-crystalline, powdered compound AFG 1. It responded to all the tests of flavonoids<sup>21,22</sup>.

#### STUDY OF THE FLAVONOID GLYCOSIDE (AFG 1):

The AFG 1 was analysed for mol. formula  $\text{C}_{33}\text{H}_{34}\text{O}_{17}$ , m.p.  $256-57^\circ$  and  $M^+ = 702$ . It reacted positively with all the tests of flavonoids.

#### UV SPECTRUM<sup>23</sup> OF THE FLAVONOID GLYCOSIDE (AFG 1):

The recorded signals of the maximum absorbance, found in various solvents are given below:

$\lambda_{\text{max}}^{\text{MeOH}}$	(nm) 257, 267(sh), 368
$\lambda_{\text{max}}^{\text{MeOH} + \text{NaOMe}}$	(nm) 293, 362 and 452 (dec.)
$\lambda_{\text{max}}^{\text{MeOH} + \text{AlCl}_3}$	(nm) 263(sh), 273, 338 and 453
$\lambda_{\text{max}}^{\text{MeOH} + \text{AlCl}_3 + \text{HCl}}$	(nm) 268, 302(sh), 365 and 422(dec.)
$\lambda_{\text{max}}^{\text{MeOH} + \text{NaOAc}}$	(nm) 258, 379 and 430(sh) (dec.)

#### INFRA-RED SPECTRUM OF THE COMPOUND AFG 1:

The IR spectrum of the compound AFG 1 showed some important peaks, which helped in interpreting important structural units with the

help of available literature<sup>23,24</sup>. These signals along with their structural assignments are given in the Table 1 (Fig 1).

TABLE 1

S.NO.	Peaks (cm <sup>-1</sup> )	Structural Assignments
1.	3400	Hydroxyl group
2.	2915	CH stretching
3.	1715	C = O group of ester
4.	1668, 1648	C = O stretching
5.	1605, 1510	Aromatic nature
6.	1380, 1370	Gem dimethyl group
7.	1280	C-O-C stretching
8.	1220	C-O-C bending
9.	1148	C-O stretching
10.	823	Two adjacent H in benzene

#### PRESENCE OF HYDROXYL GROUP (s):

In the IR spectrum of the AFG 1, a peak at 3400 cm<sup>-1</sup> suggested the presence of hydroxyl group(s) in the compound. The number of -OH groups were determined by the peracetylation of this compound with sodium acetate and acetic anhydride, which yielded an acetylated product AFG 1ac, which was analysed for

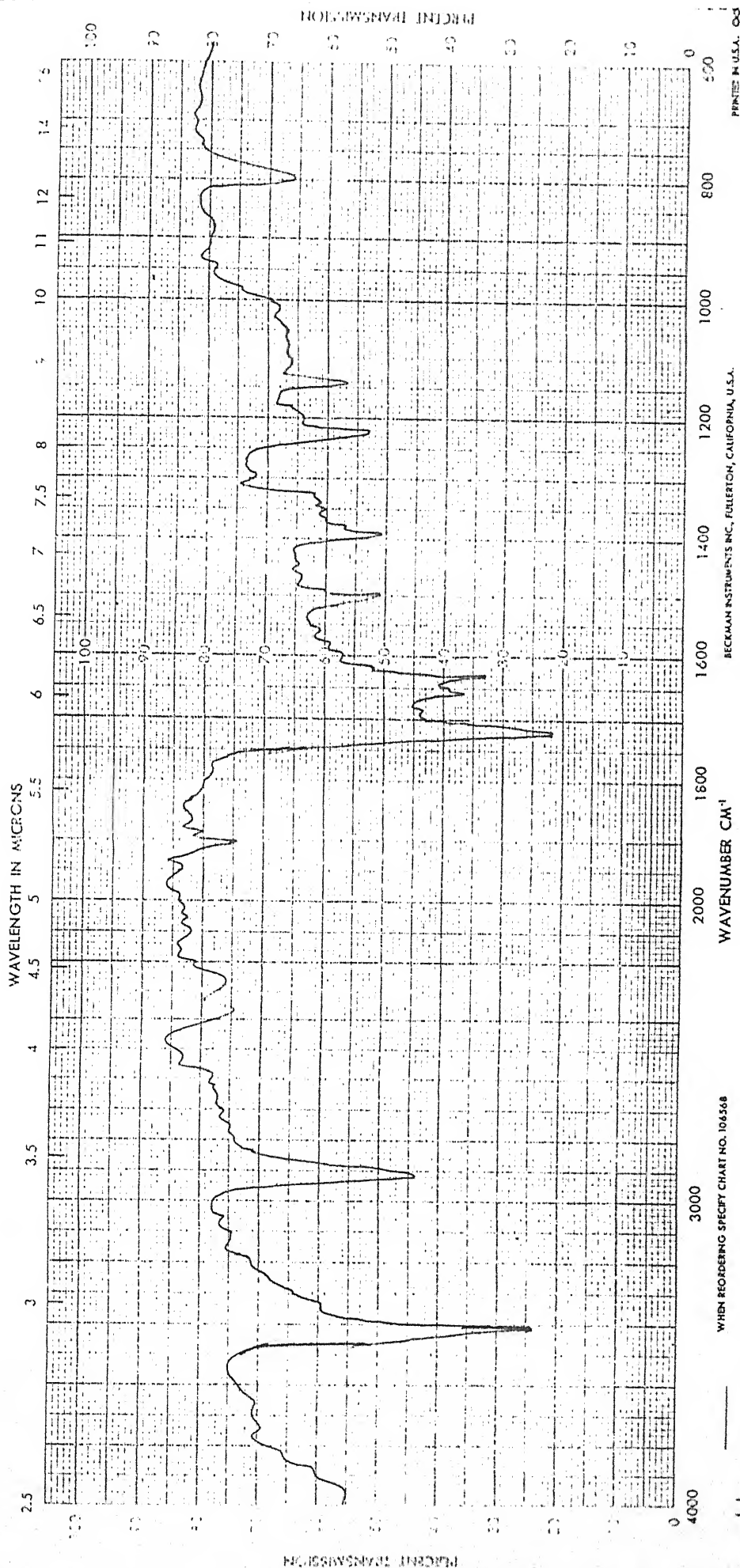


Fig I

$C_{55}H_{56}O_{28}$  and m.p. 246-48°. By Wisenberger method<sup>25</sup> (acetyl group percentage = 40.02%) eleven -OH groups were found in the compound.

Therefore, eleven out of seventeen oxygen atoms were present as hydroxyl groups in the compound AFG 1.

#### **ALKALINE HYDROLYSIS WITH SODIUM METHOXIDE:**

In the <sup>1</sup>H NMR spectrum of the compound AFG 1ac, a downfield chemical shift at  $\delta$  5.10 was an indication of the acylation in the glycoside<sup>26</sup>. Therefore, the glycoside was subjected to the alkaline hydrolysis with sodium methoxide<sup>27</sup>. The reaction mixture was neutralised with dil. AcOH. On TLC examination, two spots were shown in the solution. Then reaction mixture was concentrated under vacuum when it gave a syrupy mass, which was partitioned with solvent ether into ether soluble fraction ESC and an ether insoluble fraction AFG 2.

#### **STUDY OF ETHER SOLUBLE FRACTION (ESC):**

The solvent was removed under reduced pressure to yield a mass, which was found homogeneous by the TLC examination (Pet. ether-benzene-acetic acid 4:4:2).

The homogeneous mass was crystallised as colourless crystals and was analysed for molecular formula  $C_8H_8O_5$ , m.p. 260-261° and  $M^+$  184 (EIMS).

#### **IR SPECTRUM OF THE COMPOUND:**

The peaks obtained in the IR spectrum of the compound ESC and the structural units inferred with the help of available literature<sup>23,24,28</sup> are given in the Table 2 (Fig 2).

TABLE 2

S. No.	Wave No. $\text{cm}^{-1}$	Assignment
1.	3475	-OH group
2.	2910	C-H stretching
3.	2805	-OCH <sub>3</sub> group
4.	1705	C=O group of ester
5.	1605, 1505	Aromatic ring system
6..	1205	C-O-C bending vibration

A peak at  $3475 \text{ cm}^{-1}$  in the IR spectrum of the compound ESC suggested the presence of free -OH group(s). On acetylation ( $\text{Ac}_2\text{O}/\text{pyr.}$ ), compound ESC formed acetate with molecular formula  $\text{C}_{14}\text{H}_{14}\text{O}_8$ . On estimation of acetyl groups, 3 -OH groups (acetyl group percentage = 40.89 %) were found to be present in the compound.

By the Co TLC and comparison of the IR and  $^1\text{H}$  NMR spectra, the compound ESC was identified as methyl ester of gallic acid (I).

#### $^1\text{H}$ NMR SPECTRUM OF THE ACETYLATED DERIVATIVE COMPOUND ESC:

The  $^1\text{H}$  NMR spectrum of the acetylated derivative of the compound ESC showed some significant peaks, which were inferred with the help of available literature<sup>28</sup> and are given in the Table 3 (Fig 3).

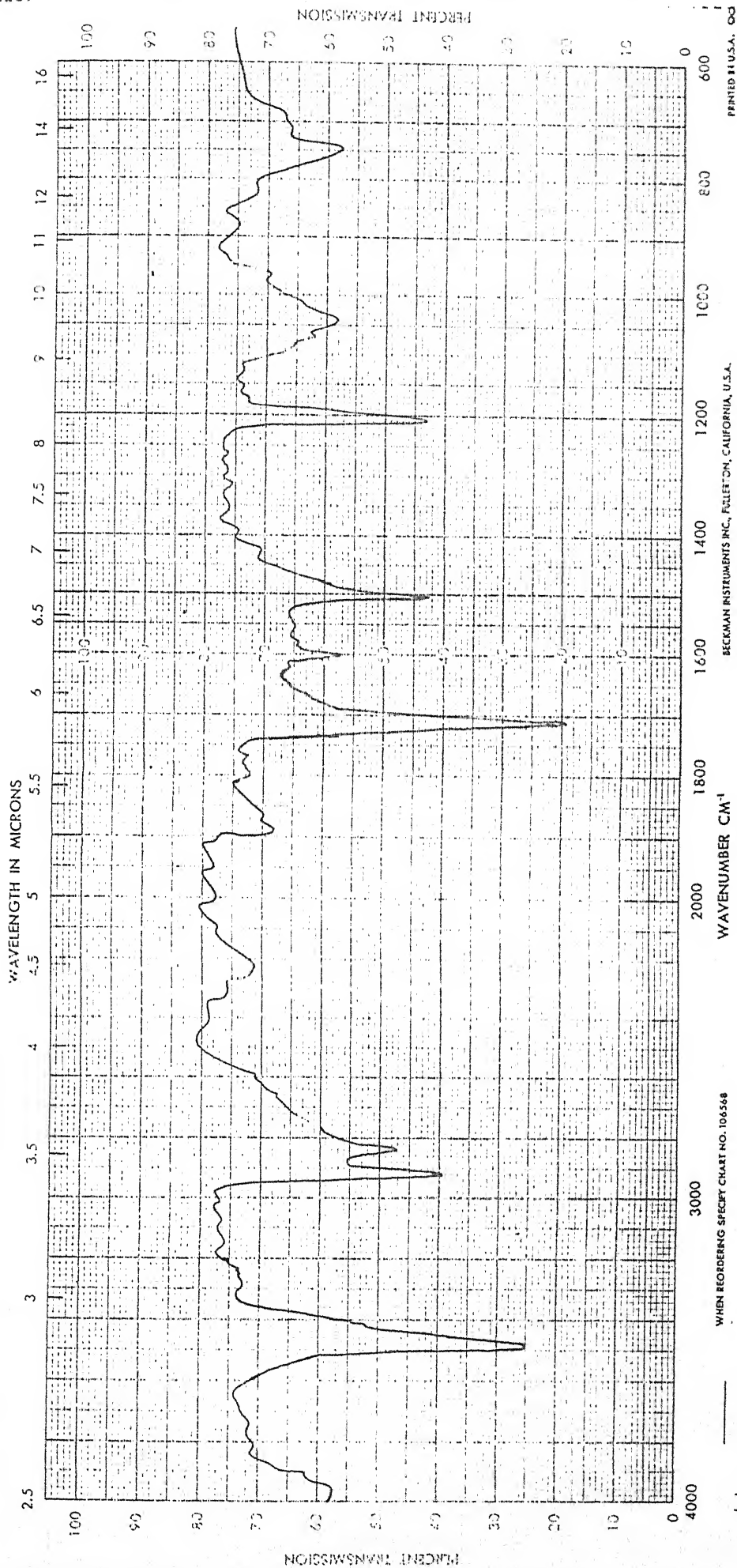


Fig 2

TABLE 3

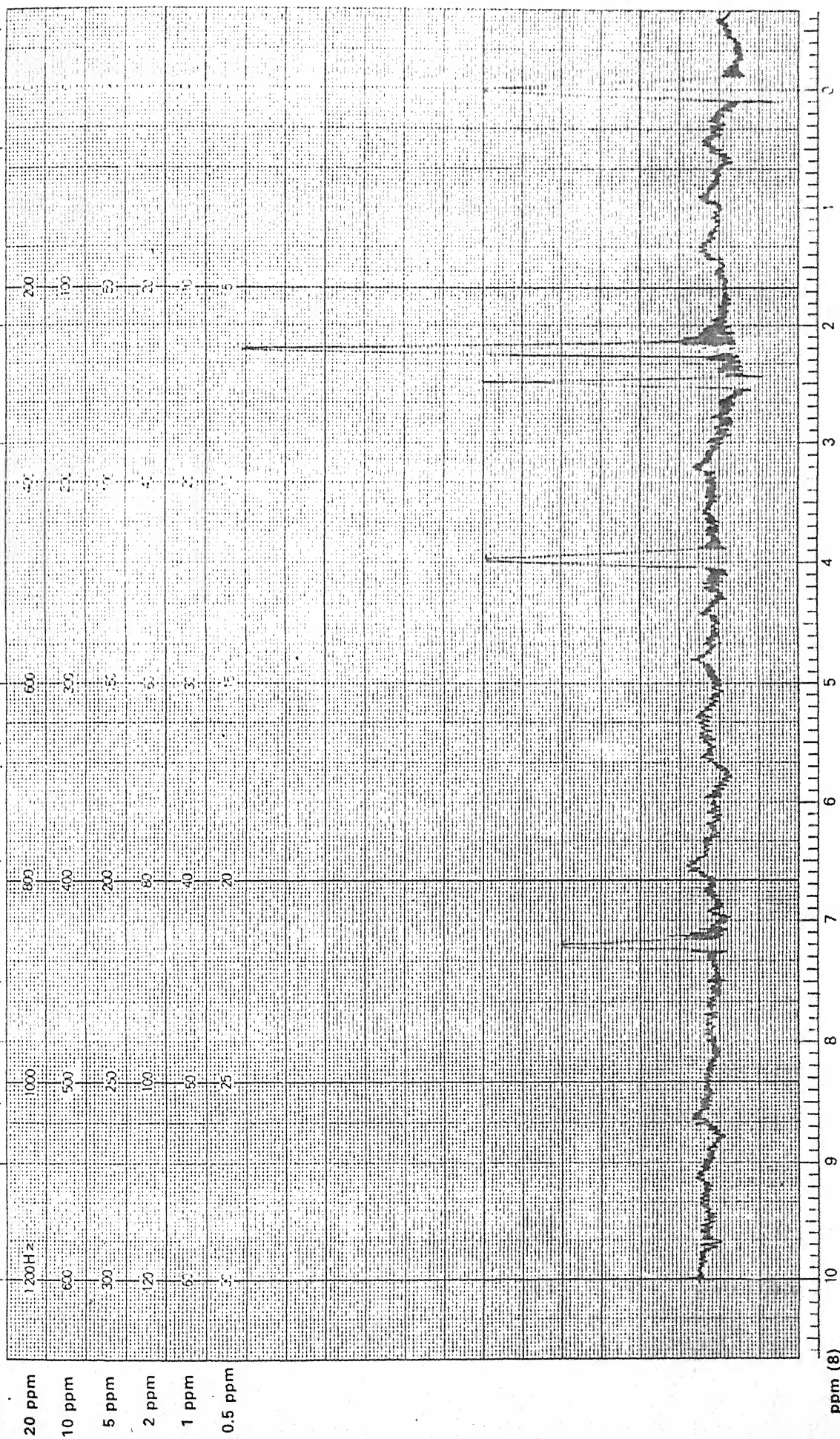
S. No.	$\delta$ Value	No. of protons	Pattern	J value (Hz.)	Assignments
1.	7.12	2	s	-	H-2''' and H-6'''
2.	3.85	3	s	-	-OCH <sub>3</sub>
3.	2.28	6	s	-	3''' and 5''' -OAc
4.	2.46	3	s	-	4''' -OAc

#### STUDY OF ETHER INSOLUBLE FRACTION (AFG 2):

The ether insoluble part was concentrated under reduced pressure to get a residue, which was found to be homogeneous on TLC examination and responded positively to the characteristic colour reactions of flavonoidal glycoside. The residue on crystallisation with acetone yielded a yellowish amorphous substance AFG 2.

The compound AFG 2 was analysed for molecular formula,  $C_{26}H_{30}O_{13}$ , m.p. 267-69° and  $M^+$  550 (EIMS).

START OF SWEEP  $\rightarrow$  H  $\rightarrow$  END OF SWEEP



LOCK POS. \_\_\_\_\_ ppm SPECTRUM AMPL. \_\_\_\_\_ SWEEP TIME \_\_\_\_\_ min NUCLEUS \_\_\_\_\_ SAMPLE \_\_\_\_\_ OPERATOR \_\_\_\_\_

LOCK POWER \_\_\_\_\_ mG FILTER \_\_\_\_\_ Sec ZERO REF. \_\_\_\_\_ DATE \_\_\_\_\_

DECOUPLE POS. \_\_\_\_\_ ppm RF POWER \_\_\_\_\_ mG END OF SWEEP \_\_\_\_\_ ppm SAMPLE TEMP. \_\_\_\_\_ °C SPECTRUM NO \_\_\_\_\_

DECOUPLE POWER \_\_\_\_\_ mG

Fig 3

## UV SPECTRUM<sup>29</sup> OF THE COMPOUND AFG 2:

The UV spectrum of the compound AFG 2, showed following wavelength of maximum absorption recorded in various solvents:

$\lambda_{\text{max}}^{\text{MeOH}}$	(nm)	258, 270(sh), 370
$\lambda_{\text{max}}^{\text{MeOH} + \text{NaOMe}}$	(nm)	290, 365 and 455 (dec.)
$\lambda_{\text{max}}^{\text{MeOH} + \text{AlCl}_3}$	(nm)	263(sh), 274, 340 and 456
$\lambda_{\text{max}}^{\text{MeOH} + \text{AlCl}_3 + \text{HCl}}$	(nm)	269, 304(sh), 367 and 421(dec.)
$\lambda_{\text{max}}^{\text{MeOH} + \text{NaOAc}}$	(nm)	257, 379 and 430(sh) (dec.)

## IR SPECTRUM OF THE COMPOUND AFG 2:

In the IR spectrum of the compound AFG 2, some important signals were observed. These peaks helped in inferring some important structural units with the help of available literature<sup>30,31</sup> and are given in the Table 4 (Fig 4).

TABLE 4

S.NO.	Peaks (cm <sup>-1</sup> )	Structural Assignments
1.	3405	Hydroxyl group
2.	2915	CH stretching
3.	1665, 1646	C = O stretching
4.	1605, 1510	Aromatic nature
5.	1382, 1372	Gem dimethyl group
6.	1280	C-O-C stretching
7.	1220	C-O-C bending
8.	1152	C-O stretching
9.	825	Two adjacent H in benzene

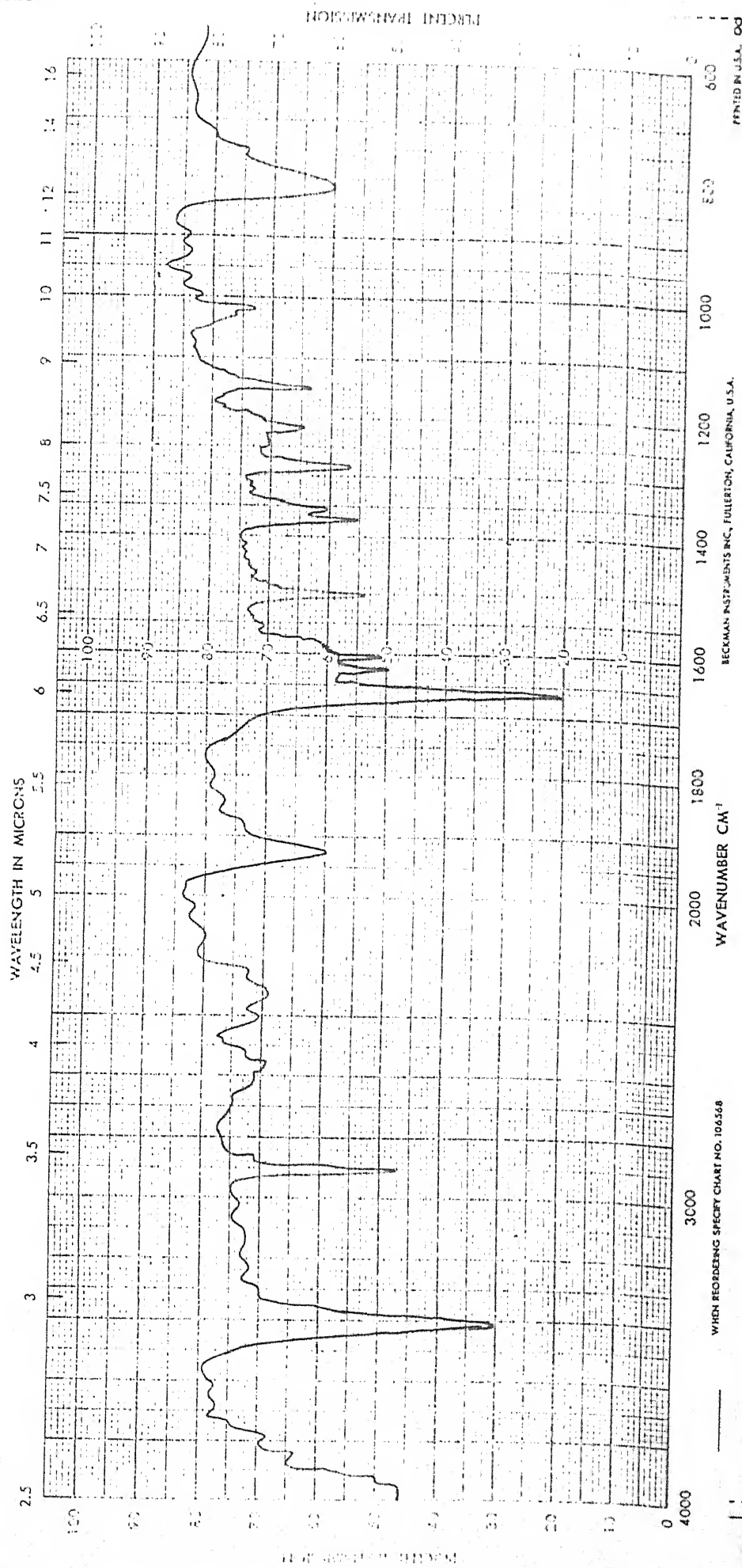


Fig 4

### **PRESENCE OF HYDROXYL GROUP (s):**

The presence of hydroxyl group(s) in the compound was evident from a peak in the IR spectrum at  $3405\text{ cm}^{-1}$ . The acetylation of the compound with  $\text{NaOAc}/\text{Ac}_2\text{O}$  yielded an acetylated derivative AFG 2ac, which analysed for the molecular formula  $\text{C}_{44}\text{H}_{48}\text{O}_{22}$ , m.p.  $258-59^\circ$ . The percentage of acetyl groups (acetyl group % = 42.15%) in the compound was determined by the Wisenberger method<sup>25</sup>, which accounted for the nine  $-\text{OH}$  groups in the compound. Thus, the existence of nine out of thirteen oxygen atoms as  $-\text{OH}$  groups was established in the compound AFG 2.

The exact structure of flavonoidal glycoside was determined by its acidic hydrolysis into aglycone and sugar, which were studied separately for structural elucidation.

### **ACID HYDROLYSIS OF THE FLAVONOIDAL GLYCOSIDE AFG 2:**

The compound AFG 2 was hydrolysed with 7% ethanolic sulphuric acid, which after filtration gave an aglycone. The hydrolysate was kept for the study of sugar.

### **STUDY OF AGLYCONE AFG 3:**

AFG 3 was analysed for the molecular formula  $\text{C}_{20}\text{H}_{20}\text{O}_8$ , m.p.  $278-79^\circ$  and  $M^+ = 388$  (EIMS). Aglycone AFG 3 gave all the colour reactions of flavonoids<sup>32</sup>.

### **UV SPECTRUM OF AGLYCONE AFG 3 :**

In the UV spectrum of the compound AFG 3, some characteristic signals with various shift reagents were observed, which are given as below:

$\lambda_{\text{max}}^{\text{MeOH}}$	(nm)	256, 268(sh), 300(sh) and 365
$\lambda_{\text{max}}^{\text{MeOH} + \text{NaOMe}}$	(nm)	248 (sh), 325 (dec.) and 418 (dec.)
$\lambda_{\text{max}}^{\text{MeOH} + \text{AlCl}_3}$	(nm)	274, 305 (sh), 334 and 458
$\lambda_{\text{max}}^{\text{MeOH} + \text{AlCl}_3 + \text{HCl}}$	(nm)	264, 302(sh), 360 and 418
$\lambda_{\text{max}}^{\text{MeOH} + \text{NaOAc}}$	(nm)	258 (sh), 283, 327 and 389 (dec.)

### IR SPECTRUM OF THE AGLYCONE AFG 3:

The aglycone AFG 3 showed important peaks in its IR spectrum. These peaks were found helpful in inferring important structural units with the help of available literature<sup>33,34</sup>. The signals and their associated structural units are given in the Table 5 (Fig 5).

TABLE 5

S.NO.	Peaks (cm <sup>-1</sup> )	Structural Assignments
1.	3405	Hydroxyl group
2.	2915	CH stretching
3.	1665, 1646	C = O stretching
4.	1605, 1510	Aromatic nature
5.	1382, 1372	Gem dimethyl group
6.	1280	C-O-C stretching
7.	1220	C-O-C bending
8.	1152	C-O stretching
9.	825	Two adjacent H in benzene

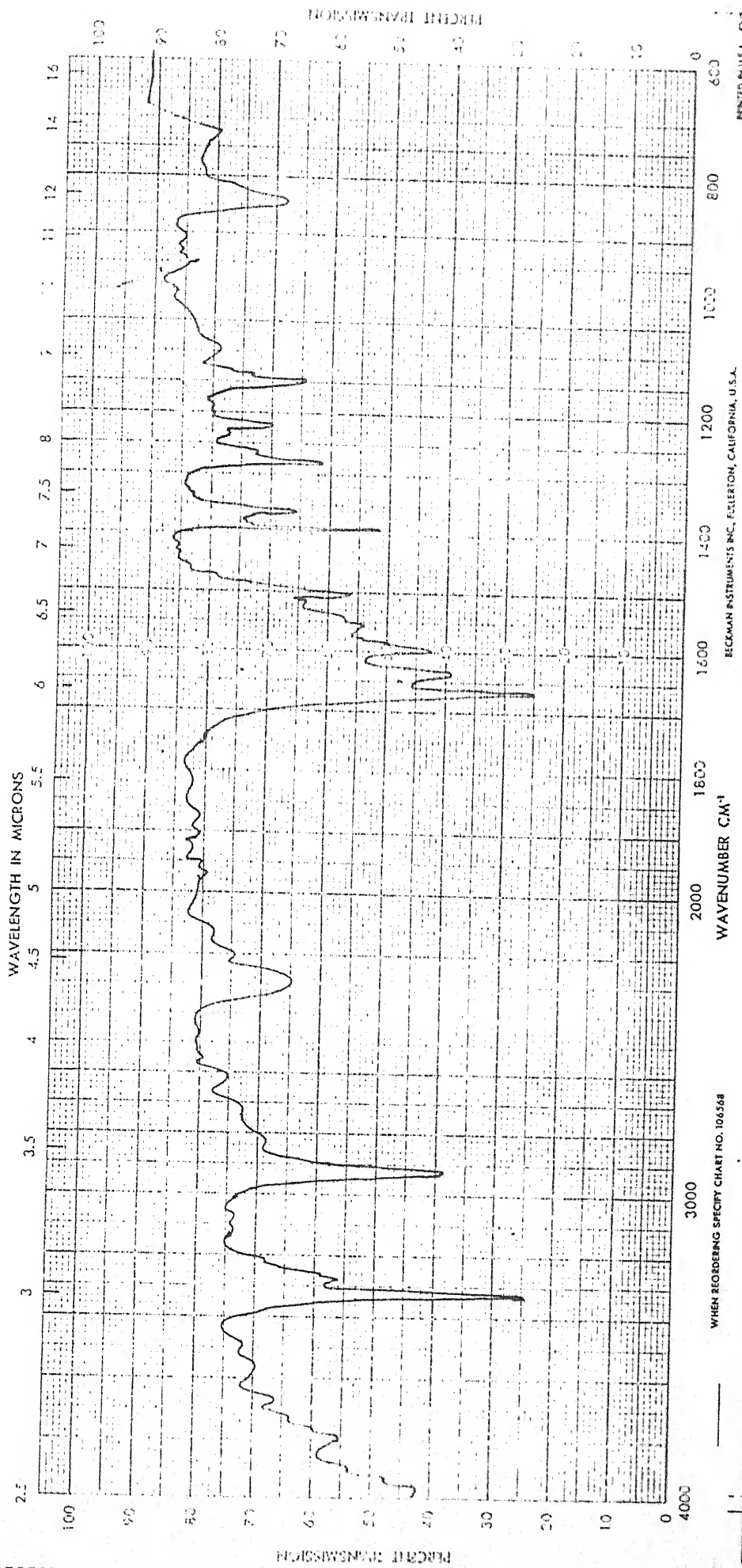


Fig 5

### **PRESENCE OF HYDROXYL GROUPS:**

The aglycone showed an IR peak at  $3405\text{ cm}^{-1}$ , which indicated the presence of hydroxyl group(s) in it. The number of hydroxyl groups were estimated by the acetylation of the compound with NaOAc/Ac<sub>2</sub>O that gave an acetylated derivative AFG 3ac, which analysed for the molecular formula C<sub>32</sub>H<sub>32</sub>O<sub>14</sub> and m.p. 270-71°. Six -OH groups were found in the compound by the Wisenberger method described by the Belcher and Godbert<sup>25</sup> (acetyl groups % = 39.82%). Thus, the presence of six out of eight oxygen atoms, as -OH groups was determined in the compound AFG 3.

### **THE POSITION OF -OH GROUPS:**

The positions of the -OH groups in the aglycone AFG 3 were determined by various shifts in the UV spectrum, <sup>1</sup>H & <sup>13</sup>C NMR, and mass spectral data.

### **HYDROXYL GROUP AT POSITION C-3 IN THE AGLYCONE :**

A 53 nm bathochromic shift in the UV spectrum of the compound AFG 3, suggested the presence of a -OH group at position C-3<sup>35</sup>. This was further corroborated by the mass spectral data of the compound AFG 3. Appearance of a M<sup>+</sup>-18 peak in the mass spectrum of the compound was a good indication of the flavonol type of nucleus<sup>36</sup>. Fragmentation pattern involved the pathway II, characteristic of flavonols, which is evident by the emergence of peaks at m/z 153 and 137.

### **HYDROXYL GROUPS AT C-5 AND C-7 IN THE AGLYCONE :**

In the UV spectrum of the aglycone a bathochromic shift of 53 nm in band I in presence of AlCl<sub>3</sub>/HCl indicated a free 5-OH group in the flavonoid<sup>35</sup>. On addition of NaOMe, appearance of a new band at 325 nm pointed towards the presence of

-OH group at C-7, which was further confirmed by the bathochromic shift of 15 nm in the band II on adding NaOAc<sup>35</sup>.

#### HYDROXYL GROUPS AT C-3' & C-4' POSITIONS :

In the UV spectrum of compound AFG 3, a second peak at 267(sh) nm suggested the presence of 3', 4' - diOH system<sup>35</sup>. In the <sup>1</sup>H NMR spectrum, signals at  $\delta$  6.80 (1H, d,  $J = 8.5$  Hz, H-5') 7.40 (1H, dd,  $J = 2.2$  & 8.5 Hz, H-6') and 7.52 (1H, d,  $J = 2.2$ , H-2') were identical to those of disubstituted B ring protons of a quercetin unit<sup>26</sup>.

#### PRESENCE OF 2''-HYDROXY-3''-METHYL BUTYL MOIETY:

In the IR spectrum of compound AFG 3, peaks at  $\nu_{\text{max}}^{\text{KBr}}$  1372 and 1382  $\text{cm}^{-1}$  indicated the presence of gem dimethyl group in it<sup>37</sup>.

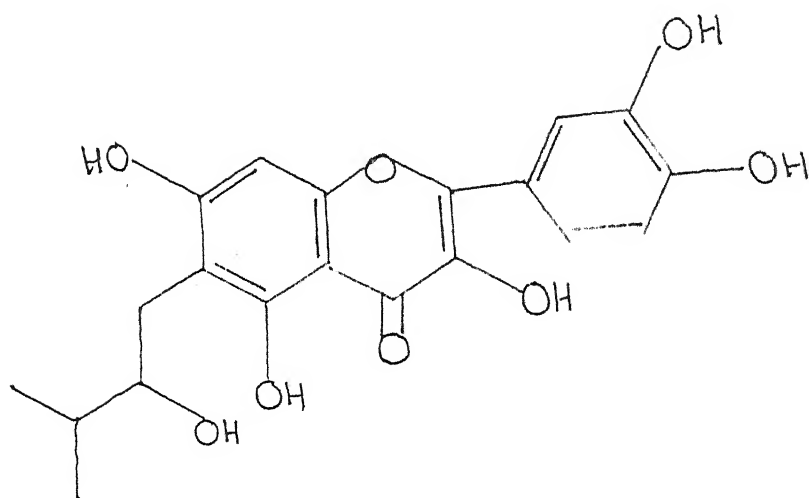
This was confirmed by the mass spectrum of the compound, and the <sup>1</sup>H NMR spectrum of the compound AFG 3, which was almost similar to that of quercetin with an exception due to the presence of 2''-hydroxy-3''-methyl butyl moiety [ $\delta$  1.63, d, 3H, H-5''; 1.69, d, 3H, H-4''; 3.10, m, 2H, H-1''; 4.23, m, 1H, H-2'' and 3.90, m, 1H, H-3'']<sup>34,38-40</sup>.

#### POSITION OF HYDROXY PRENYL SUBSTITUENT:

In the above discussion it has been proved that no position in ring B and C is unoccupied, therefore, the point of attachment of hydroxy prenyl chain can only be on ring A. A singlet at  $\delta$  6.70 (1H, s) was due to the proton of position 8 of ring A<sup>41</sup>. Therefore, in ring A also, the positions at 5,7 and 8 were occupied and only position remain vacant was at C-6, thus, it could be the point of attachment of hydroxy prenyl chain in the compound. The location of hydroxy prenyl substituent at C - 6 of aglycone AFG 3 was also assigned by the comparison of <sup>13</sup>C NMR chemical shift of C - 6 and C -

8 (107.3 and 94.3 respectively) with those for quercetin and 6 or 8 substituted 5,7 - dihydroxy flavones<sup>26&38-40</sup>. This also suggested the trisubstituted nature of ring A and confirmed the position of hydroxy prenyl substituent at C - 6.

Thus, in light of the above discussions, the following structure can be assigned to the aglycone AFG 3



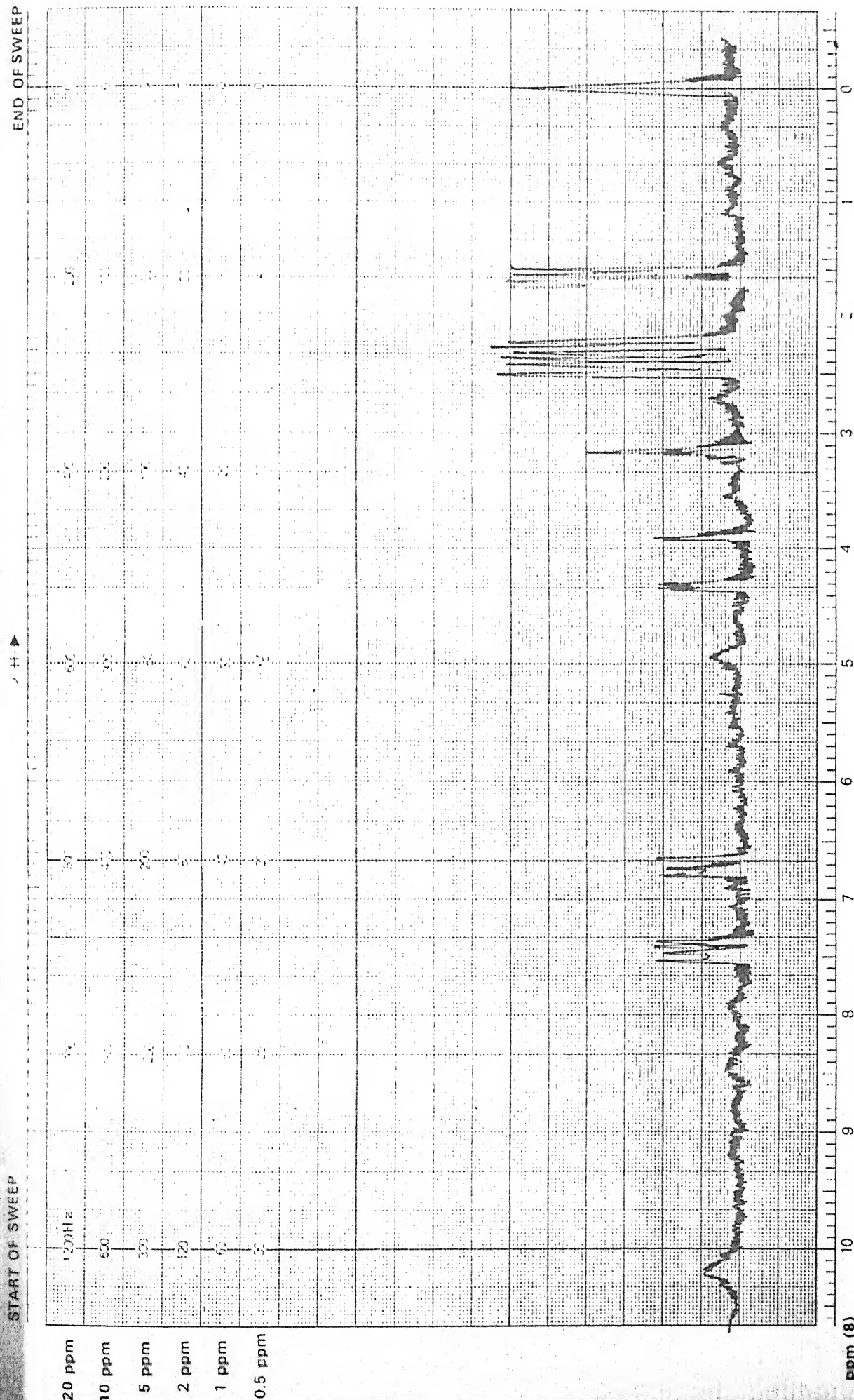
#### <sup>1</sup>H NMR SPECTRUM OF THE HEXA ACETYL DERIVATIVE OF THE AGLYCONE:

The above structure was found to be in full accordance with the <sup>1</sup>H NMR spectrum of the hexa acetyl derivative of the aglycone AFG 3. The important

signals recorded in the  $^1\text{H}$  NMR spectrum of the aglycone and the structural units inferred with the help of available literature<sup>26,32,39</sup> are given below in the Table 6 (Fig 6).

TABLE 6

S. NO.	$\delta$ VALUE	PATTERN	J VALUE IN HZ.	NO. OF PROTONS	ASSIGNMENTS
1.	7.52	d	2.2	1	H-2'
2.	7.40	dd	2.2 & 8.5	1	H-6'
3.	6.80	d	8.5	1	H-5'
4.	6.70	s	-	1	H-8
5.	4.23	m	-	1	H-2''
6.	3.90	m	-	1	H-3''
7.	3.10	m	-	2	H-1''
8.	2.47	s	-	3	5-OAc
9.	2.44	s	-	3	7-OAc
10.	2.41	s	-	3	3-OAc
11.	2.40	s	-	3	3'-OAc
12.	2.34	s	-	3	4'-OAc
13.	2.24	s	-	3	3''-OAc
14.	1.69	d	-	3	H-4''
15.	1.63	d	-	3	H-5''



LOCK POS. \_\_\_\_\_ ppm  
 LOCK POWER \_\_\_\_\_ mG  
 DECOUPLE POS. \_\_\_\_\_ ppm  
 DECOUPLE POWER \_\_\_\_\_ mG

SPECTRUM AMPL. \_\_\_\_\_ ppm  
 FILTER \_\_\_\_\_ mG  
 RF POWER \_\_\_\_\_ mG

SWEEP TIME \_\_\_\_\_ min  
 SWEEP WIDTH \_\_\_\_\_ ppm  
 END OF SWEEP 7.2 ppm

NUCLEUS \_\_\_\_\_  
 ZERO REF. \_\_\_\_\_ ppm  
 SAMPLE TEMP. \_\_\_\_\_ °C

SAMPLE \_\_\_\_\_  
 OPERATOR \_\_\_\_\_  
 DATE \_\_\_\_\_  
 SPECTRUM NO \_\_\_\_\_

### <sup>13</sup>C NMR SPECTRUM OF AGLYCON AFG 3:

The <sup>13</sup>C NMR spectrum of the compound AFG 3 showed some significant peaks, which helped in the assignment of the C skeleton of the molecule. The <sup>13</sup>C NMR spectral data and the positions assigned to the C atoms with the help of available literature<sup>26,42</sup> are given in the following Table 7.

TABLE 7

S. NO.	$\delta$ VALUE	PATTERN	ASSIGNMENTS
1.	158.6	s	C-2
2.	135.6	s	C-3
3.	179.0	s	C-4
4.	162.5	s	C-5
5.	107.3	s	C-6
6.	163.6	s	C-7
7.	94.3	d	C-8
8.	158.8	s	C-9
9.	105.3	s	C-10
10.	123.0	s	C-1'
11.	116.8	d	C-2'
12.	146.3	s	C-3'
13.	148.6	s	C-4'
14.	115.5	d	C-5'

15.	123.0	d	C-6'
16.	30.9	t	C-1''
17.	77.5	d	C-2''
18.	30.1	d	C-3''
19.	25.1	q	C-4''
20.	18.5	q	C-5''

### MASS SPECTRUM<sup>43</sup> OF THE AGLYCONE AFG 3:

The mass spectra of the compound AFG 3 was in full accordance with the structure assigned to the aglycone AFG 3. The different species obtained during the fragmentation of the molecule are shown in the **scheme I**. The different  $m/z$  values obtained in the spectra are given below:

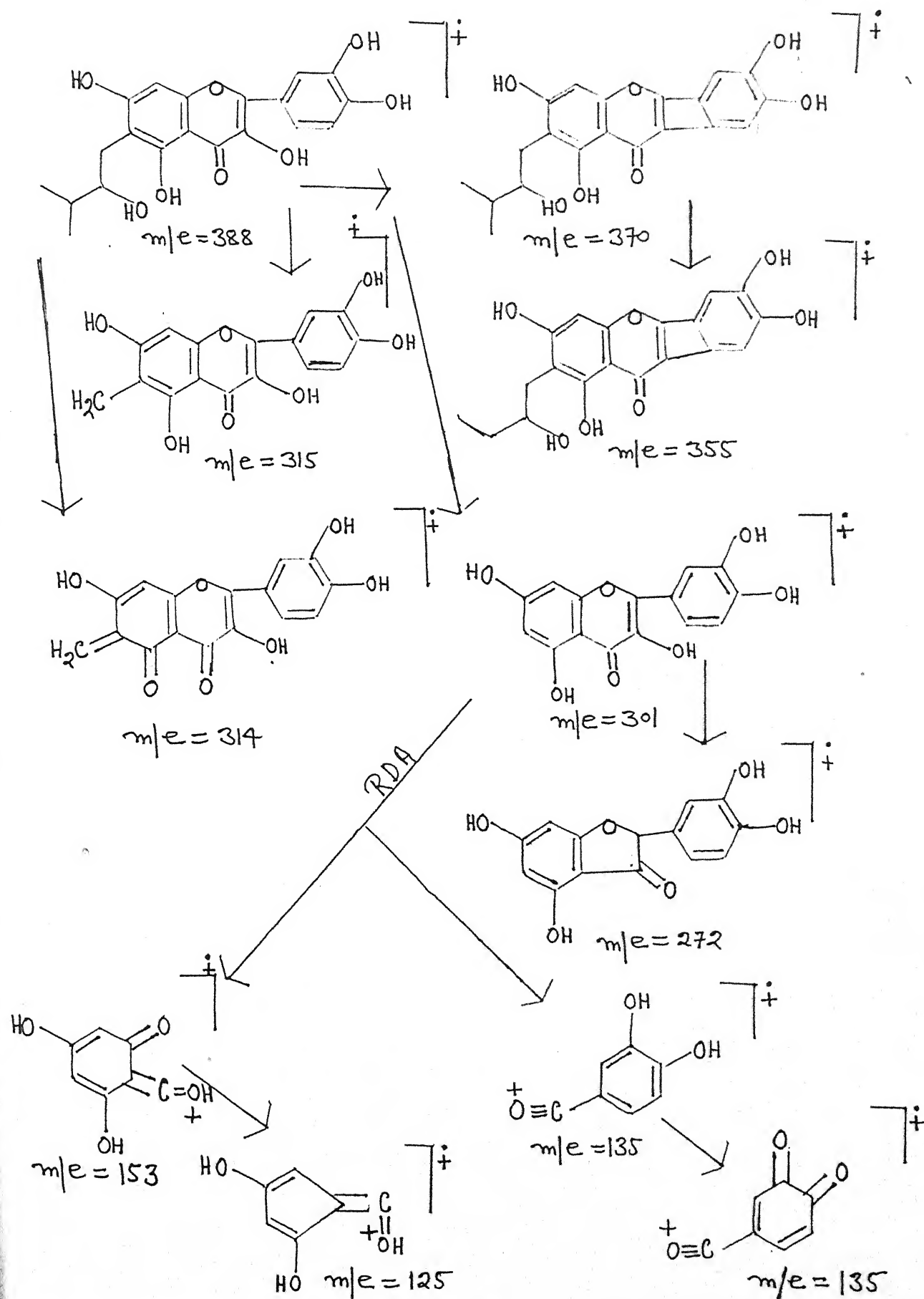
$M^+ = 388$  and  $m/z = 370, 355, 315, 314, 301, 272, 153, 137, 135$  and  $125$ .

### STUDY OF SUGAR:

After separating the aglycone, the aq. hydrolysate obtained after acid hydrolysis of AFG 2, was neutralised with barium carbonate and barium sulphate formed was separated by filtration. The filtrate on concentration gave a golden yellow mass that was found to be D-glucose by Co-PC with authentic substance.

### RATIO OF AGLYCONE AND D-GLUCOSE IN THE GLYCOSIDE AFG 2:

The ratio of aglycone and D-glucose was found 1:1 by the study of <sup>13</sup>C NMR and <sup>1</sup>H NMR spectral data.



### LINKAGE OF D-GLUCOSE WITH AGLYCONE :

Glycoside AFG 2 was subjected to permethylation followed by hydrolysis to form 2,3,4,6-tetra-O-methyl-D-glucose (Co PC and Co TLC), which showed that D-glucose was attached through its position 1 to the aglycone. This was again confirmed by the anomeric proton peak at  $\delta$  5.51 in the  $^1\text{H}$  NMR spectrum<sup>26,27</sup>

### NATURE OF THE D-GLUCOSE:

The formation of 2,3,4,6-tetra-O-methyl-D-glucose because of permethylation of compound AFG 2 also suggested the pyranose form of D-glucose.

### NATURE OF THE GLYCOSIDIC LINKAGE:

When the glycoside AFG 2 was hydrolysed by enzyme almond-emulsin<sup>27</sup>, it yielded D-glucose (confirmed by Co PC and Co TLC). This was a clear indication of  $\beta$  glycosidic linkage between D-glucose and aglycone.

### ATTACHMENT OF D-GLUCOSE TO AGLYCONE:

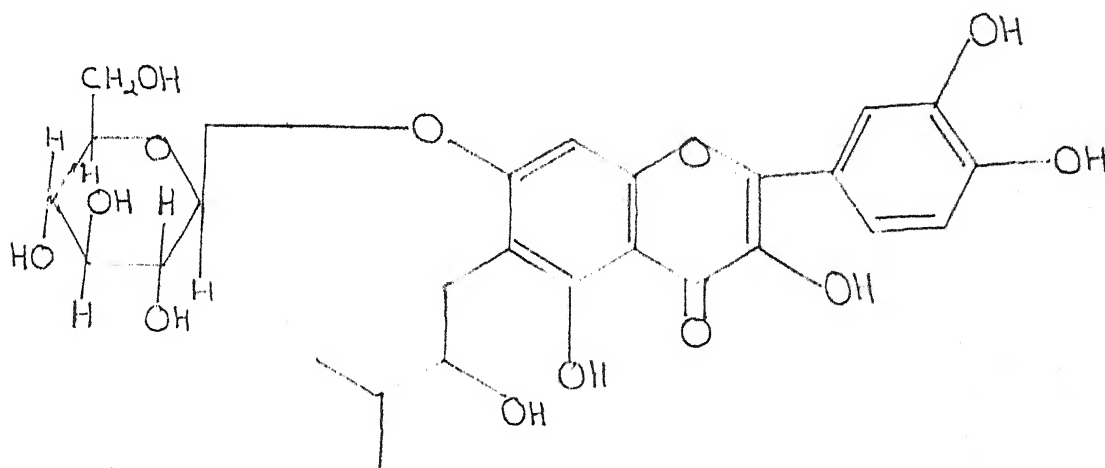
The point of attachment of D-glucose, with aglycone was ascertained by comparison of UV spectral data of aglycone with that of glycoside AFG 2 and was found to be at position 7 in the glycoside AFG 2 on the basis of the following facts:

- a). In the aglycone AFG 3, on addition of NaOMe, appearance of a new peak at 325 nm was an indication of the presence of a free 7-OH group which was absent in the glycoside AFG 2<sup>35</sup>. This clearly indicated towards the absence of free 7-OH group in the glycoside AFG 2.
- b). On addition of NaOAc, a bathochromic shift of 15 nm in the UV spectrum of the aglycone AFG 3 was another proof of the presence of 7-OH group in the aglycone. The

absence of this shift in the glycoside AFG 2, was also pointing towards the 7-O-glycosidic linkage<sup>15</sup>.

Therefore, the D-glucose was attached to the aglycone at position 7, which was also confirmed by the comparison of the UV spectrum of the aglycone and glycoside AFG 2, which gave no indication of the presence of free 7-OH group in the glycoside.

Therefore, in light of the above discussed facts, the structure of the glycoside AFG 2 can be given as 6-[2''-hydroxy-3''-methyl butyl] quercetin-7-O- $\beta$ -D-glucopyranoside, which was assigned as follows:



This structure of compound AFG 2 was further supported by the <sup>1</sup>H NMR spectrum of its nonaacetyl derivative and the <sup>13</sup>C NMR and mass spectral data of the compound.

# <sup>1</sup>H NMR SPECTRUM OF THE NONAACETYLATED DERIVATIVE OF COMPOUND AFG 2:

In the <sup>1</sup>H NMR spectrum of the nonaacetylated derivative of glycoside AFG 2, the signals obtained were in full conformity with the above structure. The significant signals obtained and the structural units inferred with the available literature<sup>26,34</sup> are given below in the Table 8 (Fig 7).

TABLE 8

S. NO.	δ VALUE	PATTERN	J VALUE IN HZ.	NO. OF PROTONS	ASSIGNMENTS
1.	6.70	s	-	1	H-8
2.	7.40	dd	2.2 & 8.5	1	H-6'
3.	6.80	d	8.5	1	H-5'
4.	7.52	d	2.2	1	H-2'
5.	2.46	s	-	3	5-OAc
6.	2.43	s	-	3	3-OAc
7.	2.37	s	-	3	4'-OAc
8.	2.41	s	-	3	3'-OAc
9.	1.63	d	-	3	H-5''
10.	1.69	d	-	3	H-4''
11.	3.92	m	-	1	H-3''
12.	4.22	m	-	1	H-2''
13.	3.12	m	-	2	H-1''

14.	2.24	s	-	3	3''-OAc
15.	5.53	d	7	1	Anomeric proton of sugar
16.	4.31-4.82	m	-	6	Protons of sugar
17.	2.50	s	-	3	6'''-OAc
18.	2.03	s	-	3	4'''-OAc
19.	2.13	s	-	3	3'''-OAc
20.	2.08	s	-	3	2'''-OAc

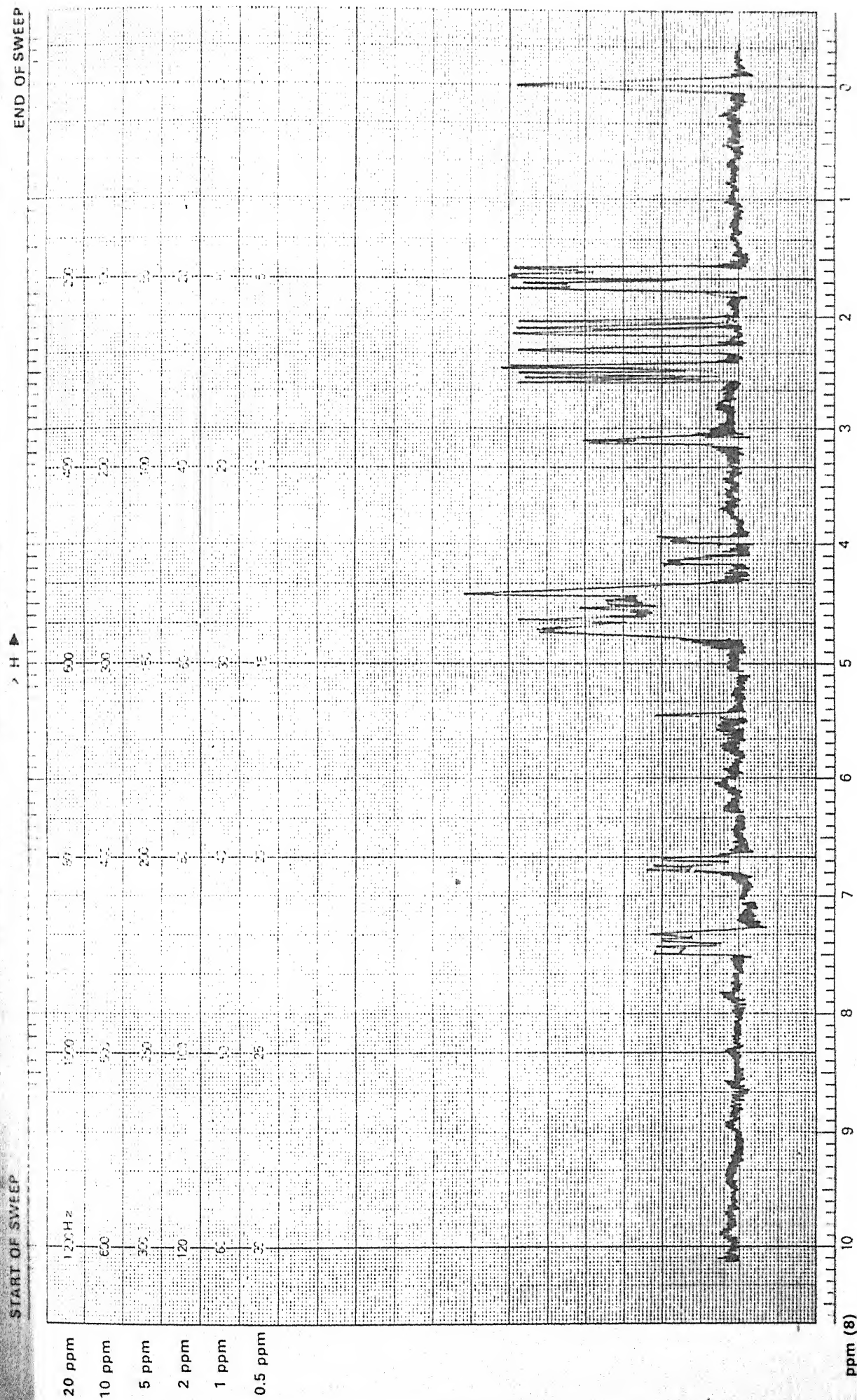
### <sup>13</sup>C NMR SPECTRUM OF COMPOUND AFG 2:

The <sup>13</sup>C NMR spectrum of compound AFG 2 showed some important peaks, which were utilised to interpret the positions of various C atoms with the help of available literature<sup>34,42</sup> These signals along with structural units inferred are given below in the Table 9.

TABLE 9

S. No.	δ	pattern	Carbon no.
VALUE			
1.	158.3	s	C-2
2.	135.4	s	C-3
3.	178.9	s	C-4
4.	162.3	s	C-5

EM-360 60 MHz NMR SPECTROMETER



LOCK POS. \_\_\_\_\_ ppm SPECTRUM AMPL. \_\_\_\_\_ min NUCLEUS \_\_\_\_\_ SAMPLE \_\_\_\_\_ OPERATOR \_\_\_\_\_

LOCK POWER \_\_\_\_\_ mG FILTER \_\_\_\_\_ Sec SWEEP WIDTH \_\_\_\_\_ ppm ZERO REF. \_\_\_\_\_ DATE \_\_\_\_\_

DECOUPLE POS. \_\_\_\_\_ ppm RF POWER \_\_\_\_\_ mG END OF SWEEP \_\_\_\_\_ ppm SAMPLE TEMP. \_\_\_\_\_ °C SOLVENT \_\_\_\_\_ SPECTRUM NO \_\_\_\_\_

DECOUPLE POWER \_\_\_\_\_ mG

Fig. 7

---

5.	107.7	s	C-6
6.	167.5	s	C-7
7.	94.8	d	C-8
8.	158.9	s	C-9
9.	105.1	s	C-10
10.	123.2	s	C-1'
11.	116.7	d	C-2'
12.	146.1	s	C-3'
13.	148.8	s	C-4'
14.	115.9	d	C-5'
15.	122.6	d	C-6'
16.	30.6	t	C-1''
17.	77.8	d	C-2''
18.	30.0	d	C-3''
19.	25.6	q	C-4''
20.	18.3	q	C-5''
21.	104.8	d	C-1'''
22.	80.1	d	C-2'''
23.	76.9	d	C-3'''
24.	72.6	d	C-4'''
25.	77.5	d	C-5'''
26.	62.1	t	C-6'''

---

### **MASS SPECTRUM<sup>43</sup> OF THE GLYCOSIDE AFG 2:**

The mass spectra of the glycoside showed some remarkable species in its fragmentation pattern, which were found to be in complete accord with its proposed structure. The different species obtained in its spectra are given as below:

[M+] = 550 and other signals at m/z 388, 370 [388-18], 355, 315, 314, 301, 272, 153, 137, 135 and 125.

### **ACID HYDROLYSIS OF GLYCOSIDE AFG 1:**

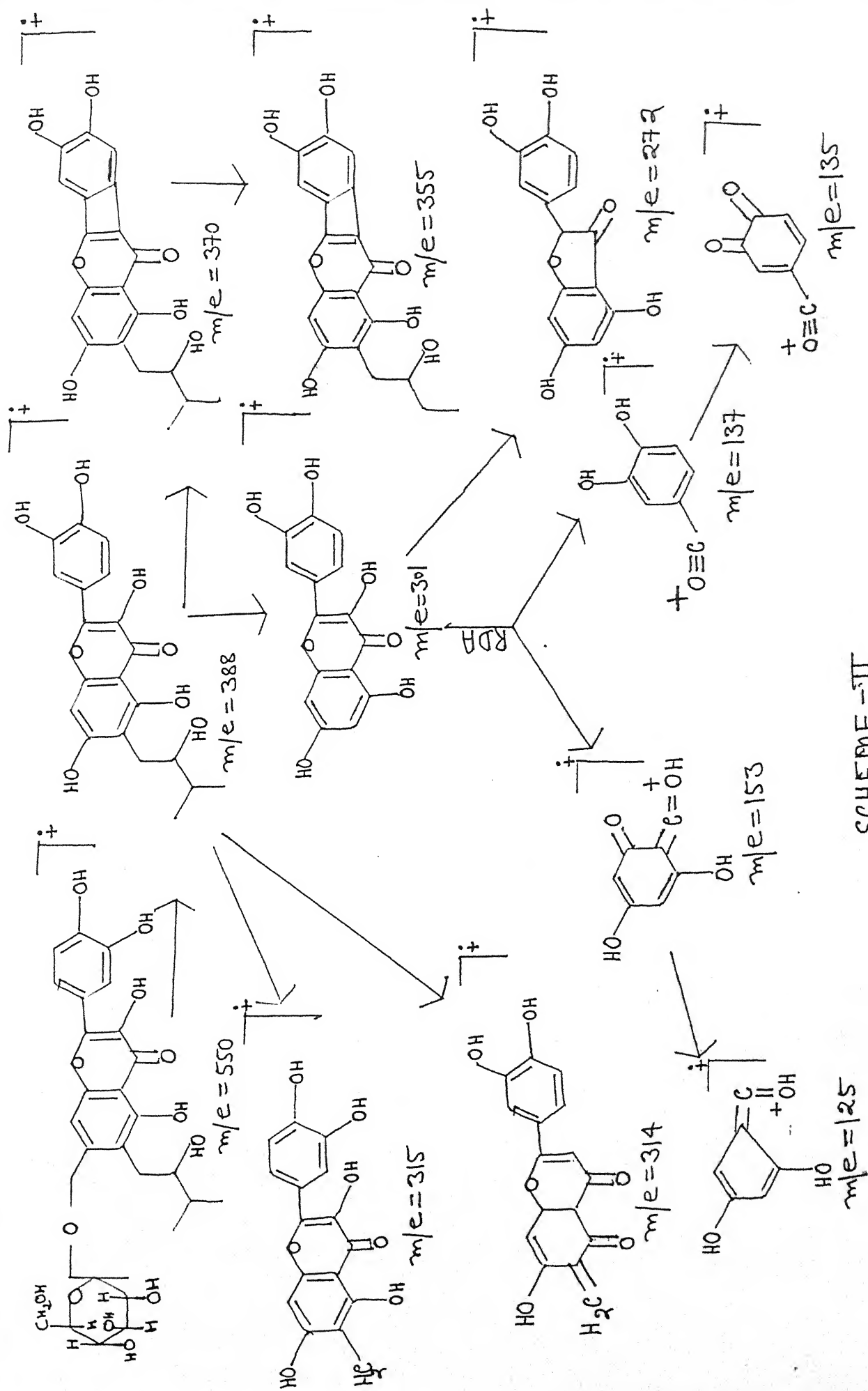
The compound AFG 1, on acid hydrolysis with 22% methanolic sulphuric acid yielded a ppt that was separated by filtration. The aqueous hydrolysate was worked up separately for the identification of sugars.

### **STUDY OF THE PRECIPITATE:**

Two compounds were shown by the TLC examination, therefore, the ppt. was subjected to column chromatography over Si gel column. The column was eluted with chloroform and methanol in various proportions.

### **STUDY OF CHLOROFORM : METHANOL 8:2 FRACTIONS:**

The R<sub>f</sub> value of eluates collected from chloroform: methanol (8:2), were same and therefore, mixed. On removal of the solvent, a yellowish mass was obtained which was found homogeneous on TLC examination. (CHCl<sub>3</sub>: MeOH 7:3). On crystallisation with acetone, this mass gave a yellowish microcrystalline powder. This was analysed for molecular formula C<sub>20</sub>H<sub>20</sub>O<sub>8</sub>, m.p. 278-79° and M<sup>+</sup> 388 (EIMS) and responded positively to the colour reactions of flavonoids. It was found identical with the



SCHEME-II

compound AFG 3 (by Co and Co TLC) formed by the acid hydrolysis of compound AFG 2.

#### STUDY OF THE CHLOROFORM: METHANOL (7:3) FRACTIONS:

The eluates from chloroform: methanol (7:3), were of similar R<sub>f</sub> value, therefore, mixed. On removing the solvent, a homogeneous compound (by TLC) was obtained after crystallisation with MeOH, which was identical with the gallic acid (by Co PC and Co TLC with authentic sample), m.p. 264-265°.

As far as point of attachment of methyl gallate to the compound was concerned, two possibilities may be possible.

1. Either the methyl gallate might be attached with the aglycone AFG 3 unit.
2. Or it might be attached with the sugar unit.

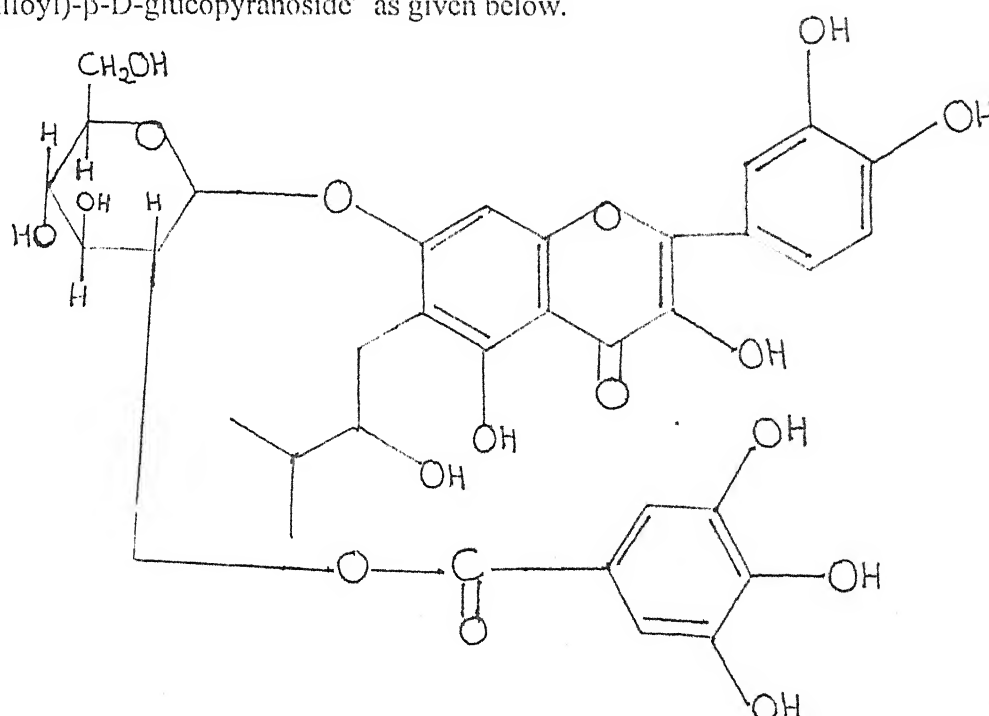
Since all the -OH groups in the compound AFG 1 were free except the 7-OH group, which was involved in the glycosidic linkage, therefore, the only possibility left was that the gallic acid was attached to the glucose unit. In the <sup>1</sup>H NMR spectrum of the glycoside AFG 1, a downfield chemical shift at  $\delta$  5.10 was an indication of acylation in the D-glucose unit<sup>26</sup>.

#### POSITION OF ATTACHMENT OF GALLIC ACID UNIT TO THE D-GLUCOSE:

The attachment of C-1''' of glucose with C-7 of aglycone has already been discussed. To establish the position of attachment of gallic acid to the glucose unit, the glycoside AFG 1 was permethylated<sup>27</sup>, followed by acid hydrolysis to yield 2,4,6-tri-O-methyl D-glucose (identified by Co PC and Co TLC). This indicated that C-2'' of glucose moiety was involved in the acylation.

Further, the attachment of gallic acid with C-2''' of glucose was proved by the  $^1\text{H}$  NMR spectrum of the acetylated derivative of the acylated glycoside (AFG 1). In the  $^1\text{H}$  NMR spectrum, the chemical shifts at  $\delta$  5.51(d, 1H and  $J = 7$  Hz., anomeric proton of glucose) and  $\delta$  5.10 (1H, dd,  $J = 7.7$  Hz., C-2''' proton of sugar) confirmed the acylation at C-2''.

Keeping all these facts in consideration, the structure of acylated glycoside (AFG 1) can be given as "6-[2''-hydroxy-3''-methyl butyl] quercetin -7-O-(2'''-galloyl)- $\beta$ -D-glucopyranoside" as given below.



This structure was further supported by the  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and mass spectral data.

#### $^1\text{H}$ NMR SPECTRUM OF UNDECAACETATE OF ACYLATED GLYCOSIDE AFG 1:

The chemical shifts obtained in the  $^1\text{H}$  NMR spectrum and the structural units inferred with the help of literature<sup>31,44</sup> are given below in the Table 10 (Fig 8).

TABLE 10

S. NO.	$\delta$ VALUE	NO. OF PROTONS	PATTERN	J VALUE (HZ.)	ASSIGNM- ENT
1.	7.52	1	d	2.2	H-2'
2.	7.40	1	dd	2.2 & 8.5	H-6'
3.	6.80	1	d	8.5	H-5'
4.	6.70	1	s	-	H-8
5.	2.47	3	s	-	5-OAc
6.	2.41	3	s	-	3-OAc
7.	2.40	3	s	-	3'-OAc
8.	2.35	3	s	-	4'-OAc
9.	4.23	1	m	-	H-2''
10.	3.90	1	m	-	H-3''
11.	3.10	2	m	-	H-1''
12.	1.69	3	d	-	H-4''
13.	1.63	3	d	-	H-5''
14.	2.23	3	d	-	3''-OAc
15.	5.51	1	d	7.0	Anomeric proton of sugar.
16.	5.10	1	dd	7.7	C-2'''
17.	4.30-4.84	5	m	-	Protons of

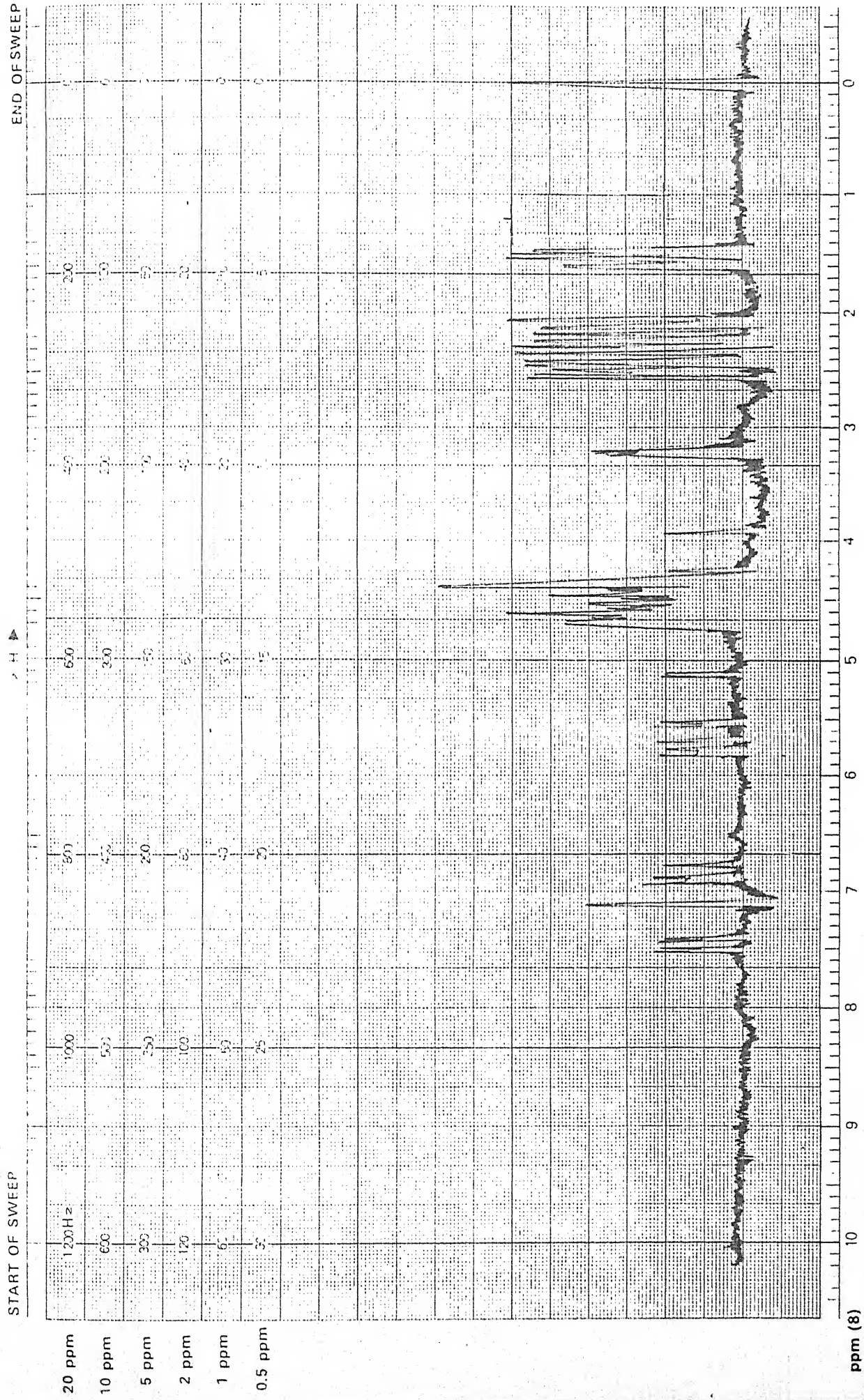
					sugar
18.	2.51	3	s	-	6'''-OAc
19.	2.15	3	s	-	3'''-OAc
20.	2.01	3	s	-	4'''-OAc
21.	7.16	2	s	-	Galloyl, H-2'''and H-6'''
22.	2.49	3	s	-	Galloyl, 4'''-OAc
23.	2.30	6	s	-	3'''and 5''' -OAc

### 13 C NMR SPECTRUM OF THE COMPOUND AFG 1:

The signals obtained in the 13 C NMR spectrum of the compound AFG 1 were utilised to interpret the C skeleton of the compound with the help of literature and are given below in the Table 11.

TABLE 11

S. NO.	$\delta$ VALUE	PATTERN	ASSIGNMENT
1.	158.4	s	C-2
2.	135.8	s	C-3
3.	178.7	s	C-4



LOCK POS. \_\_\_\_\_ ppm SPECTRUM AMPL. \_\_\_\_\_ SWEEP TIME \_\_\_\_\_ min NUCLEUS \_\_\_\_\_ SAMPLE \_\_\_\_\_ OPERATOR \_\_\_\_\_

LOCK POWER \_\_\_\_\_ mG FILTER \_\_\_\_\_ Sec SWEEP WIDTH \_\_\_\_\_ ppm ZERO REF. \_\_\_\_\_ DATE \_\_\_\_\_

DECOUPLE POS. \_\_\_\_\_ ppm RF POWER \_\_\_\_\_ mG END OF SWEEP \_\_\_\_\_ ppm SAMPLE TEMP. \_\_\_\_\_ °C SOLVENT \_\_\_\_\_ SPECTRUM NO \_\_\_\_\_

DECOUPLE POWER \_\_\_\_\_ mG

Fig 8

4.	162.6	s	C-5
5.	107.5	s	C-6
6.	167.8	s	C-7
7.	94.5	d	C-8
8.	158.6	s	C-9
9.	105.4	s	C-10
10.	122.9	s	C-1'
11.	116.4	d	C-2'
12.	145.8	s	C-3'
13.	148.2	s	C-4'
14.	115.6	d	C-5'
15.	122.9	d	C-6'
16.	30.8	t	C-1''
17.	77.4	d	C-2''
18.	30.1	d	C-3''
19.	25.3	q	C-4''
20.	18.1	q	C-5''
21.	104.5	d	C-1'''
22.	74.1	d	C-2'''
23.	78.3	d	C-3'''
24.	72.3	d	C-4'''
25.	77.2	d	C-5'''

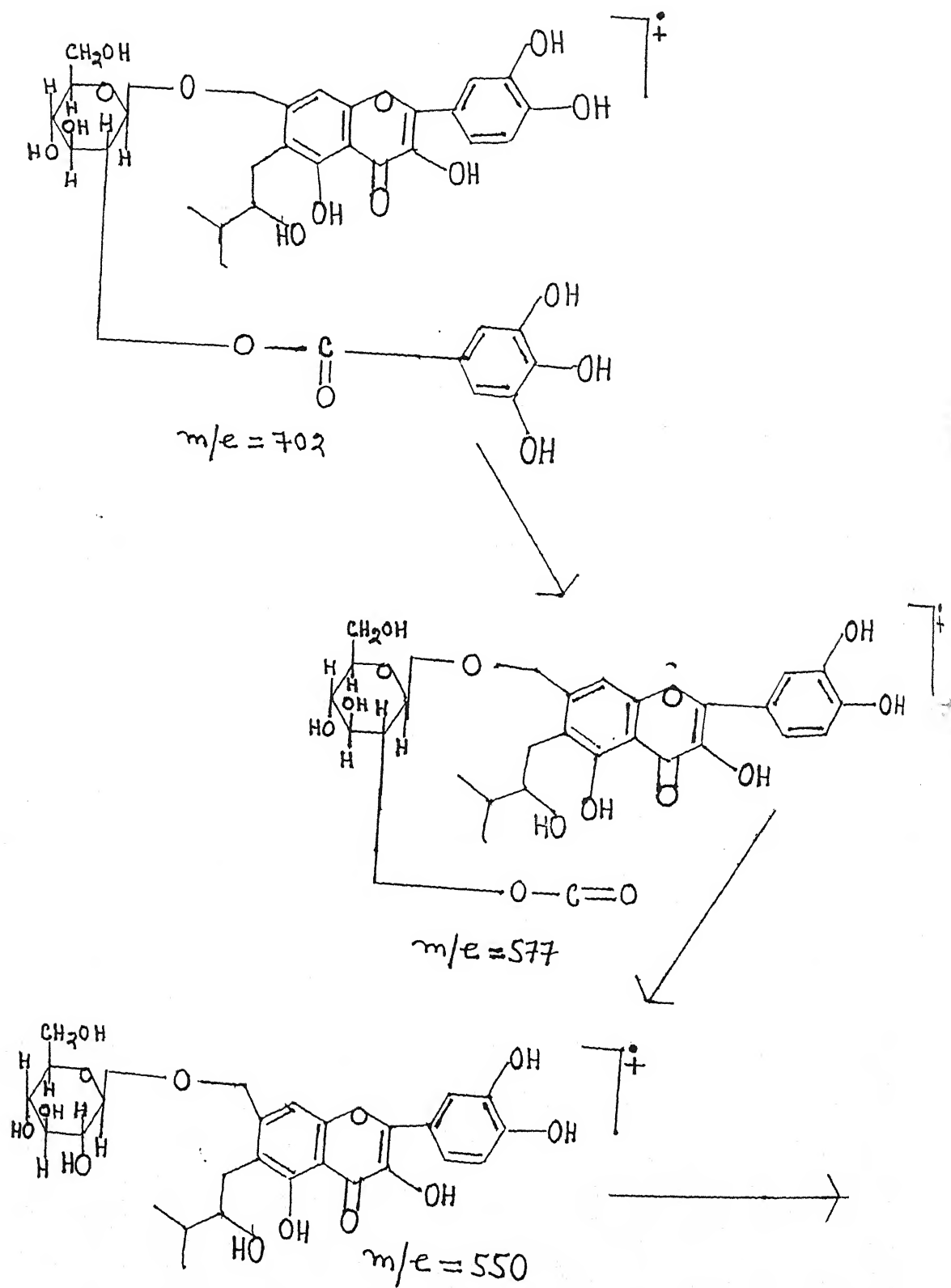
26.	62.5	t	C-6'''
27.	121.1	s	C-1'''
28.	110.7	d	C-2''' & C-6'''
29.	146.5	s	C-3''' & C-5'''
30.	140.1	s	C-4'''
31.	168.4	s	C-7'''

#### MASS SPECTRUM<sup>45</sup> OF THE GLYCOSIDE AFG 1:

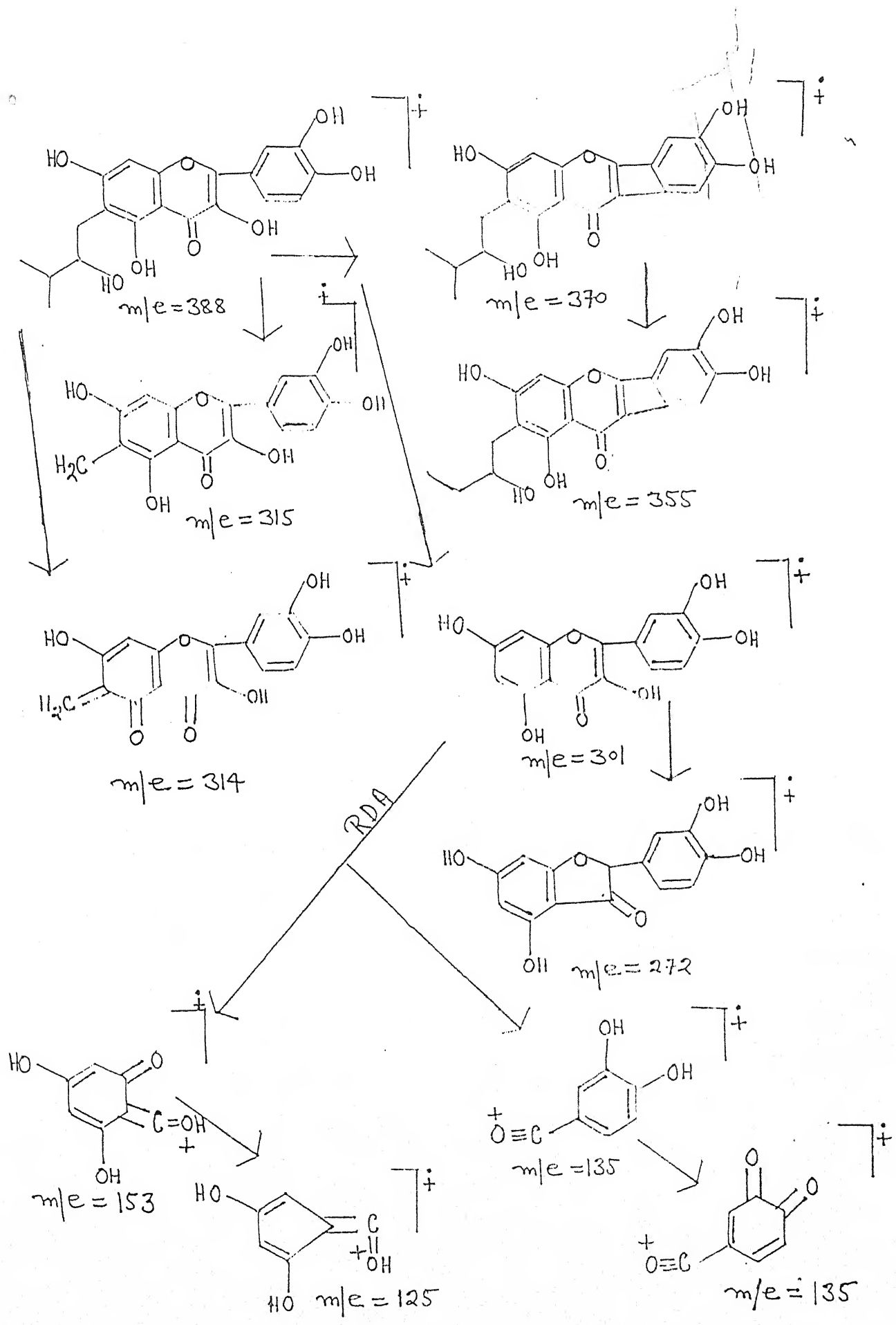
In the electron impact mass spectrum of the acylated glycoside AFG 1, various fragment peaks were obtained, which are as given below:

$M^+ = 702$ ,  $m/z$  577, 550, 388, 370, 355, 315, 314, 301, 272, 153, 137, 135 and 125.

The scheme III shows various species assigned to fragments, which, confirmed the structure of the compound AFG 1 as: "6-[2''-hydroxy-3''-methyl butyl] quercetin -7-O- (2'''-galloyl)- $\beta$ -D-glucopyranoside".



SCHEME - III



## EXPERIMENTAL

### EXTRACTION OF THE ACYLATED FLAVONOID GLYCOSIDE (AFG):

The aerial parts of the *Kickxia ramosissima* (3.0 Kg.) were collected from the adjoining regions and were authenticated by the Botany Department of this college. This plant material was air dried, finely powdered and soxhlet extracted with 95% methanol. The methanol extract was concentrated and dissolved in cold water. The aq. solution was then successively partitioned with n hexane, benzene, chloroform and ethyl acetate. The ethyl acetate soluble part (500 ml.) was concentrated under reduced pressure to get a brown viscous mass, which was subjected to column chromatography over a Si gel column (5 x 90 cm). The column was eluted with  $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$  with increasing polarity. The  $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$  (4:6) part was homogeneous on TLC examination. The solvent was concentrated under reduced pressure and the residue was crystallised with methanol to get the compound AFG 1 (2.05 g.).

### COLUMN CHROMATOGRAPHY:

Length of the column	90 cm.
Diameter of the column	5.0 cm.
Weight of the Silica gel	150 g.
Weight of the crude product	4.12 g.

TABLE 12

S. No.	Fraction no.	Eluant collected	Remarks
		50 ml. each	
1.	1-8	CHCl <sub>3</sub> : CH <sub>3</sub> OH 9:1	Sticky mass
2.	9-16	CHCl <sub>3</sub> : CH <sub>3</sub> OH 6:4	Not remarkable
3.	17-24	CHCl <sub>3</sub> : CH <sub>3</sub> OH 5:5	mixture
4.	25-32	CHCl <sub>3</sub> : CH <sub>3</sub> OH 4:6	Single spot (AFG 1)
5.	33-40	CHCl <sub>3</sub> : CH <sub>3</sub> OH 3: 7	mixture
6.	40-41	CHCl <sub>3</sub> : CH <sub>3</sub> OH 1:9	Single spot (will be studied in Ch. 3)

**STUDY OF THE COMPOUND AFG 1:**

The yellow coloured microcrystalline powdered compound AFG 1 was soluble in ethyl acetate and acetone but insoluble in petroleum ether and benzene. It analysed for molecular formula C<sub>33</sub>H<sub>34</sub>O<sub>17</sub>, m.p. 256-57°, molecular weight 702 (EIMS).

**ELEMENTAL ANALYSIS:**

Found	Calculated
C = 56.44%	56.41%
H = 4.86%	4.84%
Molecular weight = 702[EIMS]	
Molecular formula = C <sub>33</sub> H <sub>34</sub> O <sub>17</sub> .	

### ACETYLATION OF THE COMPOUND AFG 1:

Compound AFG 1 (100 mg.), fused sodium acetate (800 mg.) and acetic anhydride (5 ml.) were taken in a flask fitted with air condenser, and heated on a oil bath at 130° for 6 hours. The reaction mixture was poured in cold water after cooling. A thick white ppt. was obtained, which was extracted with solvent ether. The ethereal layer was washed with water and sodium bicarbonate solution. Finally the ethereal layer was dried over anhydrous sodium sulphate and the ether was evaporated. The residue was crystallised from methanol as white needles (60 mg.), analysed for  $C_{55}H_{56}O_{28}$ , m.p. 246-48°,  $M^+$  1164.

### ELEMENTAL ANALYSIS:

Found	Calculated
C = 56.66%	56.70%
H = 4.81%	4.81%
Molecular weight = 1164 [EIMS]	Molecular formula = $C_{55}H_{56}O_{28}$
Acetyl group percentage = 40.02%	

### ALKALINE HYDROLYSIS OF GLYCOSIDE AFG 1:

The glycoside AFG 1(1.2 g.) was hydrolysed by taking it in methanol in a 250 ml. round bottomed flask, and reacted with calculated amount of 2% sodium methoxide solution (20 ml.) and kept overnight. The reaction mixture was neutralised with acetic acid. After removal of the solvent, it gave a syrupy mass, which was partitioned with dry ether into ether soluble and ether insoluble fractions.

### STUDY OF THE ETHER SOLUBLE PART ESC:

On evaporation of solvent under reduced pressure, a homogeneous mass was obtained which on crystallisation from methanol afforded colourless crystalline compound (130 mg.), analysed for molecular formula  $C_8H_8O_5$ , m.p. 260-261° and  $M^+$  184 (EIMS).

#### ELEMENTAL ANALYSIS:

Found	Calculated
C = 52.48%	52.17%
H = 4.28%	4.35%
Molecular weight = 184 [EIMS]	Molecular formula = $C_8H_8O_5$

#### ACETYLATION OF THE ESC:

The compound ESC was acetylated in the same manner as described for the AFG 1.

#### ELEMENTAL ANALYSIS:

Found	Calculated
C = 54.55%	54.19%
H = 4.37%	4.52%
Molecular weight = 310 [EIMS]	Molecular formula = $C_{14}H_{14}O_8$
Acetyl group % = 40.89 %)	

### STUDY OF ETHER INSOLUBLE PART AFG 2:

The removal of solvent from ether insoluble fraction under reduced pressure yielded a yellowish amorphous substance (1.05 g.), which on crystallisation from acetone: MeOH (1:1) gave a microcrystalline compound. It analysed for  $C_{26}H_{30}O_{13}$ .

m.p. 267-69° , M+ 550. It gave a positive Molisch's test and responded positively with  $\text{FeCl}_3$  test.

#### ELEMENTAL ANALYSIS:

Found	Calculated
C = 56.77%	56.73%
H = 5.43%	5.45%
Molecular weight = 550 [EIMS]	Molecular formula = $\text{C}_{26}\text{H}_{30}\text{O}_{13}$

#### ACETYLATION OF THE AFG 2:

The compound AFG 2 was acetylated in the same manner as described for the AFG 1.

#### ELEMENTAL ANALYSIS:

Found	Calculated
C = 56.86%	56.90%
H = 5.19%	5.17%
Molecular weight = 928 [EIMS]	Molecular formula = $\text{C}_{44}\text{H}_{48}\text{O}_{22}$
Acetyl group % = 42.15 %	

#### ACID HYDROLYSIS OF THE GLYCOSIDE AFG 2:

The 750 mg. of compound was refluxed with 7% alcoholic  $\text{H}_2\text{SO}_4$  on a water bath for eight hours. After adding 50 ml. of water to the reaction mixture, the alcohol was removed by distilling under reduced pressure, when it yielded aglycone as a ppt. that was removed by filtration. The hydrolysate was neutralised with  $\text{BaCO}_3$  and was filtered to remove  $\text{BaSO}_4$ . The filtrate was concentrated under reduced pressure to a golden yellow mass that was examined by Co PC with authentic sample using n-Butanol:

acetic acid: water (4:1:5) as a solvent system and aniline hydrogen phthalate as a spraying reagent. This investigation proved the presence of D-glucose (Rf. value 0.20).

#### STUDY OF AGLYCON AFG 3:

The aglycone AFG 3 was a yellowish microcrystalline powder (500 mg.) with m.p. 278-79° and analysed for molecular formula  $C_{20}H_{20}O_8$ ,  $M^+$  388.

#### ELEMENTAL ANALYSIS OF COMPOUND AFG 3:

Found	Calculated
C = 61.89%	61.86%
H = 5.14%	5.16%
Molecular weight = 388 [EIMS]	Molecular formula = $C_{20}H_{20}O_8$

#### ACETYLATION OF THE AFG 3:

The compound AFG 3 was acetylated in the same manner as described for the AFG 1.

#### ELEMENTAL ANALYSIS:

Found	Calculated
C = 60.08%	60.0%
H = 5.02%	5.0%
Molecular weight = 640 [EIMS]	Molecular formula = $C_{32}H_{32}O_{14}$
Acetyl group % = 39.82%	

#### SCHINODA TEST:

Few crystals of the compound were dissolved in the 2 drops of EtOH and to this solution, Mg powder was added followed by the addition of 5M HCl. On viewing against a white background, red colour appeared.

## STUDY OF SUGAR HYDROLYSATE:

The sugar hydrolysate obtained after the acid hydrolysis was neutralised with barium carbonate and the ppt. of Barium sulphate obtained was filtered off. The filtrate after concentration under vacuum gave a golden yellow mass, which was examined on PC (Rf. 0.20) [n-BuOH: AcOH Water]. This study showed only one spot that was developed into brownish coloured spot by spraying it with aniline hydrogen phthalate. This compound was identified as D-glucose that was confirmed by the Co PC with authentic sample.

## PERMETHYLATION OF THE AFG 2:

The glycoside AFG 2 (50 mg.), MeI (5.0 ml.), silver oxide (150 mg.) and dimethyl formamide were taken at room temperature in a conical flask. After filtering the reaction mixture, residue was washed with dimethyl formamide. The filtrate was concentrated under reduced pressure to get a residue, which was dissolved in methanol. A syrupy mass was obtained on further removal of solvent, which was hydrolysed with 22%  $\text{H}_2\text{SO}_4$ . The aq. hydrolysate obtained after filtration was neutralised with barium carbonate and barium sulphate formed was filtered off. The filtrate after concentration was subjected to Co PC, using n- BuOH: AcOH: Water (4:1:5, v/v) as solvent system and aniline hydrogen phthalate as visualising agent (Rf. 0.62).

The method was repeated in a similar manner with acylated glycoside (AFG 1).

## ACIDIC HYDROLYSIS OF THE GLYCOSIDE AFG1:

The glycoside (500 mg.) was dissolved in 25 ml. of methanol and 100 ml. of 22%  $\text{H}_2\text{SO}_4$  was added to it. The reaction mixture was refluxed on a water bath for

4 hours, then cooled. The solvent was removed by concentration under reduced pressure, to obtain a crystalline ppt.

This ppt was examined on TLC using Si gel plates and chloroform: methanol (8:2) as solvent system. The plates were developed by 10% H<sub>2</sub>SO<sub>4</sub>, as visualising agent, to show the two spots (R<sub>f</sub> value 0.52 and 0.74).

#### COLUMN CHROMATOGRAPHY OF THE RESIDUE:

Weight of the compound	300mg
Length of the column	90 cm.
Diameter of the column	3.0 cm.
Weight of the Si gel	130 mg.

TABLE 13

S. No.	Fraction No.	Eluant	TLC	Remark
	(Vol of each 50 ml.)			
1.	1-10	CHCl <sub>3</sub> :CH <sub>3</sub> OH (9:1)	Nil	Sticky mass
2.	11-26	CHCl <sub>3</sub> :CH <sub>3</sub> OH (8:2)	Single spot	Aglycone AFG1
3.	27-40	CHCl <sub>3</sub> :CH <sub>3</sub> OH (7:3)	Single spot	Gallic acid
4.	41-65	CHCl <sub>3</sub> :CH <sub>3</sub> OH (5:5)	Nil	Nil

#### STUDY OF CHLOROFORM: METHANOL (8:2):

The fractions 11-26 showed single spot on TLC examination and so combined. After evaporating the solvent, the residue was crystallised with acetone to get a yellowish microcrystalline powder (AFG 3, 150 mg.) m.p. 278-79° and was analysed for molecular formula  $C_{20}H_{20}O_8$ ,  $M^+$  388. This compound has already been discussed in detail.

#### STUDY OF CHLOROFORM: METHANOL (7:3):

The fractions 27-40 were of same Rf. value and so combined. After evaporation of the solvent, it yielded a colourless crystalline compound, m.p. 264-265°. By co TLC with authentic sample, it was found identical with gallic acid, using n BuOH: formic acid: Water (4:1:5) as solvent system on Si gel plates. (Rf. value 0.71).

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### CHAPTER III

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ISOLATION AND STUDY OF A NOVEL FLAVONE GLYCOSIDE, "8-PRENYL-  
CHRYSOERIOI,-4'-O- $\beta$ -D-XYLOPYRANOSYL- (1 $\rightarrow$ 2)- $\alpha$ -L-  
ARABINOPYRANOSYL- (1 $\rightarrow$ 6)- $\beta$ -D-GALACTOPYRANOSIDE FROM *KICKXIA*  
*RAMOSISSIMA* (WALL.) JANCHEN SYN. *LINARIA RAMOSISSIMA* (FAMILY –  
SCROPHULARIACEAE)".

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The detailed description of the plant *Kickxia ramosissima* has been given in the Chapter 2 of this thesis.

#### ISOLATION OF THE FLAVONOID GLYCOSIDE:

Air-dried and finely powdered aerial parts (3.0 kg.) of *Kickxia ramosissima* were soxhlet extracted with 95% methanol. The extract was concentrated under reduced pressure to get a dark brown viscous mass, which was dissolved in cold water. The solution in water was partitioned with n hexane, benzene, chloroform and ethyl acetate. The n hexane, benzene and chloroform extracts were very small in quantity, thus, they could not be studied.

#### STUDY OF ETHYL ACETATE SOLUBLE FRACTION:

The ethyl acetate soluble part was concentrated under reduced pressure to obtain a dark brown viscous material and subjected to column chromatography over Si gel. The column was eluted with  $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$  with increasing polarity. The  $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$  (4:6) part gave a homogeneous compound, AFG 1, which was discussed in the Chapter 2 of this thesis. The  $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$  (1:9) part also showed a single spot on TLC examination. Thus,  $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$  (1:9) part was concentrated and crystallised with methanol to yield a yellow coloured micro-crystalline powdered compound FG 1. It responded to all the tests of flavonoids<sup>1,2</sup>.

#### STUDY OF THE FLAVONOIDS GLYCOSIDE (FG 1):

The FG 1 was analysed for mol. formula  $\text{C}_{37}\text{H}_{46}\text{O}_{19}$ , m.p. 308-09° and  $M^+ = 794$ . It responded positively with all the tests of flavonoids. In the UV spectrum of the compound FG 1, peaks at 268 and 342 nm, corroborated the presence of flavonoidal nucleus<sup>3</sup>.

## UV SPECTRUM<sup>4</sup> OF THE FLAVONOID GLYCOSIDE (FG 1):

The recorded signals of the maximum absorbance, found in various solvents, in the UV spectrum of the compound FG 1, are given below:

$\lambda_{\text{max}}^{\text{MeOH}}$	(nm)	243, 246 (sh), 268, 290 (sh) and 342.
$\lambda_{\text{max}}^{\text{MeOH} + \text{NaOMe}}$	(nm)	270, 320 and 365.
$\lambda_{\text{max}}^{\text{MeOH} + \text{AlCl}_3}$	(nm)	260, 278, 294, 361 and 386.
$\lambda_{\text{max}}^{\text{MeOH} + \text{AlCl}_3 + \text{HCl}}$	(nm)	258, 278, 291(sh), 346 and 382(sh).
$\lambda_{\text{max}}^{\text{MeOH} + \text{NaOAc}}$	(nm)	272, 319 and 359.
$\lambda_{\text{max}}^{\text{MeOH} + \text{NaOAc/H}_3\text{BO}_3}$	(nm)	268 and 340.

## INFRA-RED SPECTRUM OF THE COMPOUND FG 1:

Some characteristic peaks emerged in the IR spectrum of the compound FG 1 which helped in interpreting the important structural units with the help of available literature<sup>4,5</sup>, are given below in the Table 1 (Fig 1).

TABLE 1

S.NO.	Peaks (cm <sup>-1</sup> )	Structural Assignments
1.	3425	Hydroxyl group
2.	2985	CH stretching
3.	2820	-OCH <sub>3</sub> group
4.	1670, 1645	C = O stretching
5.	1600, 1505	Aromatic nature
6.	1385, 1368	Gem dimethyl group
7.	1285	C-O-C stretching
8.	1218	C-O-C bending

9.		1145	C-O stretching
10.		828	Two adjacent H in benzene

#### PRESENCE OF HYDROXYL GROUP (s):

The presence of hydroxyl group(s) in the compound was established due to a peak at  $3425\text{ cm}^{-1}$  in the IR spectrum of the FG 1. The peracetylation of this compound with sodium acetate and acetic anhydride to yield an acetylated product FG 1ac, which was analysed for  $\text{C}_{57}\text{H}_{66}\text{O}_{29}$   $\text{M}^+$  1214 and m.p  $327\text{-}28^\circ$ , helped in determining the number of  $\text{-OH}$  groups. Ten  $\text{-OH}$  groups were found in the compound by the Wisenberger method as described by the Belcher and Godbert (acetyl group percentage  $=34.94\%$ )<sup>6,7</sup>.

Therefore, the nature of ten out of nineteen oxygen atoms was established as hydroxyl group in the compound FG 1.

#### PRESENCE OF METHOXYL GROUP(s):

The IR spectrum of the compound, FG 1, exhibited a peak at  $\nu^{\text{KBr}}_{\text{max}}$   $2820\text{ cm}^{-1}$ , which was a clear indication of the presence of  $\text{-OCH}_3$  group(s). The presence of one methoxyl group was determined by the Zeisel's method<sup>8</sup>.

#### PRESENCE OF SUGAR(s):

The compound FG 1 showed positive Molisch's test, which suggested the glycosidic nature of the compound.

Thus, a tentative structure of the compound FG 1 can be given as below:

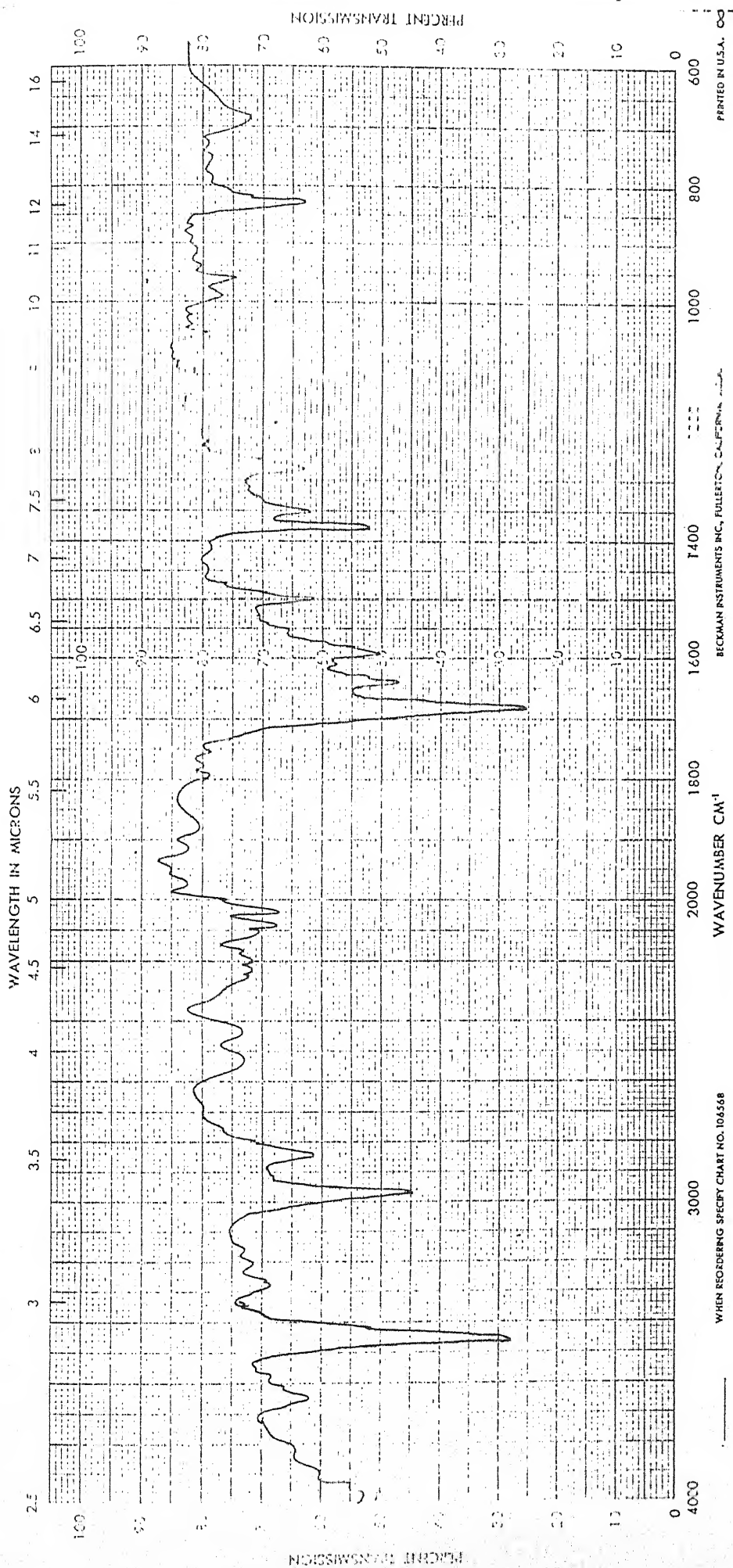
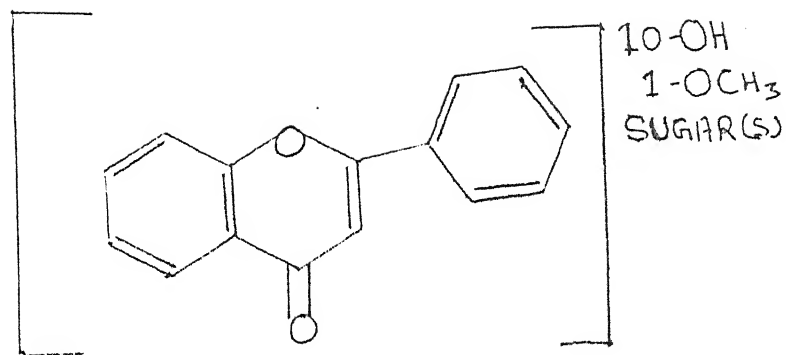


Fig. 1



### ACID HYDROLYSIS OF THE FLAVONOIDAL GLYCOSIDE FG 1:

The compound FG 1 was hydrolysed with 7% ethanolic sulphuric acid, which after filtration gave an aglycone. The hydrolysate was kept for the study of sugar.

### STUDY OF AGLYCONE FG 2:

The aglycone FG 2 was analysed for the molecular formula C<sub>21</sub>H<sub>20</sub>O<sub>6</sub>, m.p. 290-92° and M<sup>+</sup> = 368 (EIMS). Aglycone FG 2 gave all the colour reactions of flavonoids.

### UV SPECTRUM OF AGLYCONE FG 2:

The UV<sup>9</sup> spectrum of the compound FG 2 showed some characteristic signals with various shift reagents and are given as below:

$\lambda_{\text{max}}^{\text{MeOH}}$	(nm)	241, 251(sh), 269 and 346.
$\lambda_{\text{max}}^{\text{MeOH} + \text{NaOMe}}$	(nm)	264, 278 (sh), 328 (sh) and 402.
$\lambda_{\text{3max}}^{\text{MeOH} + \text{AlCl}_3}$	(nm)	263, 274, 295, 364 (sh), and 391.
$\lambda_{\text{max}}^{\text{MeOH} + \text{AlCl}_3 + \text{HCl}}$	(nm)	258, 274, 293, 352 and 388.
$\lambda_{\text{max}}^{\text{MeOH} + \text{NaOAc}}$	(nm)	269, 322 and 394.
$\lambda_{\text{3max}}^{\text{MeOH} + \text{NaOAc} / \text{H}_3\text{BO}_3}$	(nm)	266 and 348.

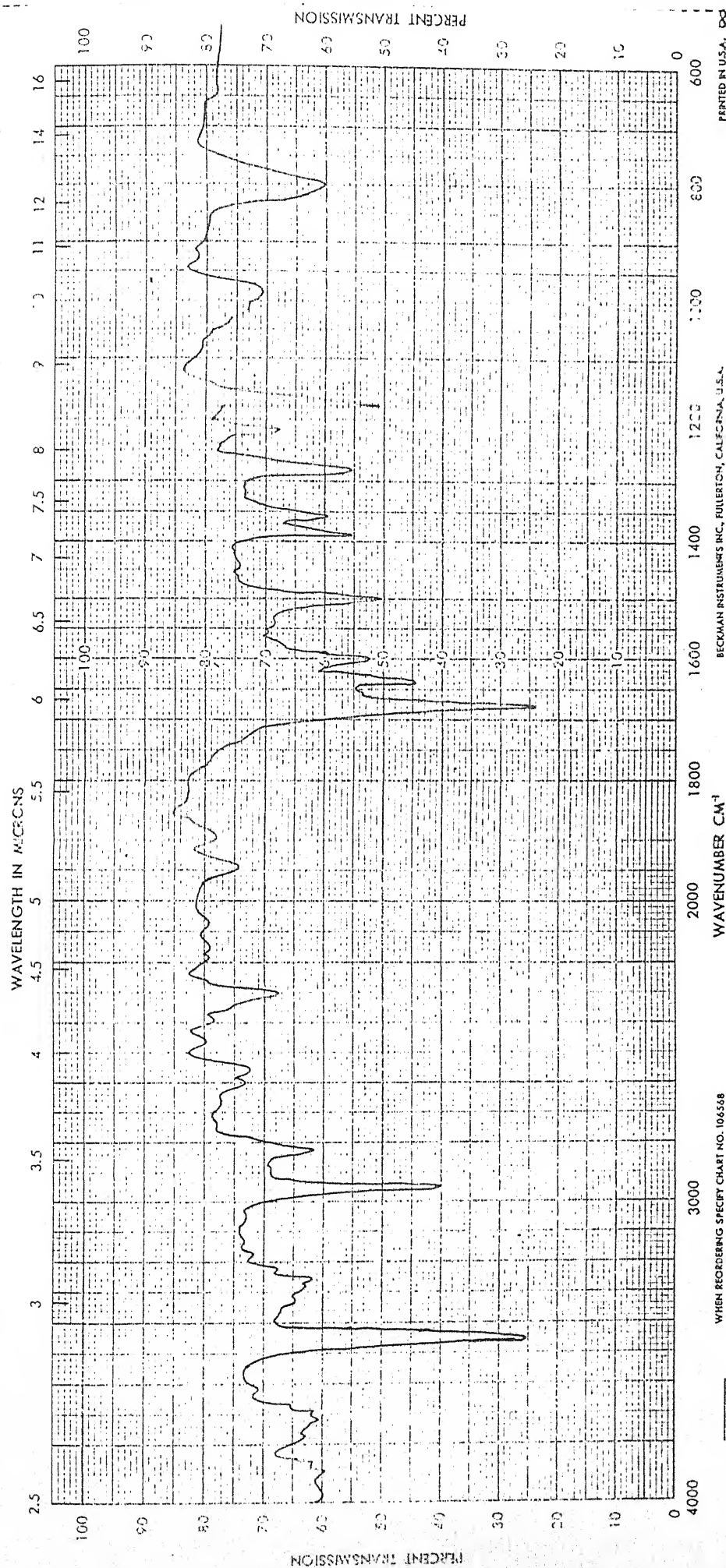


Fig. 2

## IR SPECTRUM OF THE AGLYCONE FG 2:

In the IR spectrum of the aglycone FG 2, some characteristic peaks were recorded, which were utilised in interpreting various structural units present in the compound with the help of available literature<sup>10,11</sup>. The signals and their associated structural units are given in the Table 2 (Fig 2).

TABLE 2

S.NO.	Peaks (cm <sup>-1</sup> )	Structural Assignments
1.	3428	Hydroxyl group
2.	2980	CH stretching
3.	2825	-OCH <sub>3</sub>
4.	1668, 1642	C = O stretching
5.	1610, 1500	Aromatic nature
6.	1386, 1374	Gem dimethyl group
7.	1282	C-O-C stretching
8.	1226	C-O-C bending
9.	1149	C-O stretching
10	821	Two adjacent H in benzene

### PRESENCE OF HYDROXYL GROUPS:

The IR spectrum of the aglycone recorded a peak at 3428 cm<sup>-1</sup>, which was due to the presence of hydroxyl group(s) in it. On acetylation of the compound with NaOAc/Ac<sub>2</sub>O, it gave an acetylated derivative FG 2ac, which analysed for the molecular formula C<sub>27</sub>H<sub>26</sub>O<sub>9</sub>, M<sup>+</sup>494 and m.p. 285-86°. By applying the method of Wisenberger, described by the Belcher and Godbert on the compound FG 2ac, three -OH groups were

found to be present in the compound, (acetyl group percentage = 25.19%). Thus, the presence of three out of six oxygen atoms, as -OH groups was determined in the compound FG 2.

#### THE POSITION OF -OH GROUPS:

The various shifts in the UV spectrum and the  $^1\text{H}$  &  $^{13}\text{C}$  NMR, and mass spectral data helped in ascertaining the positions of the -OH groups in the aglycone FG 2.

#### HYDROXYL GROUP AT C-4' POSITIONS:

In the UV spectrum of the compound, on adding NaOMe a stable bathochromic shift of 56 nm without decrease in intensity, was an evidence of the presence of a -OH group at C-4' position in the ring B<sup>12</sup>.

#### HYDROXYL GROUPS AT C-5 AND C-7 IN THE AGLYCONE:

A bathochromic shift of 42 nm in band I in presence of  $\text{AlCl}_3/\text{HCl}$  in the UV spectrum of the aglycone was due to the presence of a free 5-OH group in the flavonoid<sup>12</sup>. On adding NaOAc, a bathochromic shift of 28 nm in the band II suggested the presence of a -OH group at C-7, which was further corroborated by the appearance of a new band at 328 nm on adding NaOMe<sup>13</sup>.

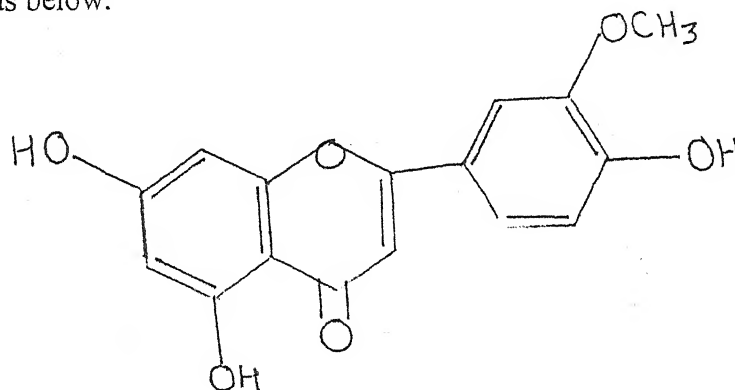
#### PRESENCE OF METHOXYL GROUP:

A peak at  $\nu_{\text{max}}^{\text{KBr}}$  2825  $\text{cm}^{-1}$  in the IR spectrum of the aglycone FG 2, suggested the presence of -OCH<sub>3</sub> group(s) in the compound. By the Zeisel's method of methoxyl group estimation, the presence of one methoxyl group was confirmed in the compound.

### POSITION OF METHOXYL GROUP:

Two singlets at  $\delta 6.26$  and  $\delta 6.48$  accounting for one proton each in the  $^1\text{H}$  NMR spectrum of the triacetylated derivative of the aglycone were due to the protons at H-3<sup>14</sup> and H-6<sup>15</sup>. Again the  $^1\text{H}$  NMR spectrum of the FG 2 was similar to that of the disubstituted B ring protons of the chrysoeriol [ $\delta 7.82$ , 1H, d,  $J = 1.5$  Hz., H-2'; 6.82, 1H, d,  $J = 8.5$  Hz., H-5'; 7.49, 1H, dd, 1.5 & 8.5 Hz., H-6']<sup>9,16</sup>. Thus, from the above discussion it is evident that only positions left vacant for the attachment of the methoxyl group in this compound are either at C-8 in the ring A or at C-3' in the ring B. As UV spectrum of the compound, FG 2 was almost similar to that of the chrysoeriol, thus, the position of the methoxyl group was assigned at C-3'. A sharp singlet at  $\delta 3.88$  integrating for 3 protons was a confirmation of the presence of the one  $-\text{OCH}_3$  group at position 3' of the ring B<sup>17</sup>.

Due to the above discussion, a tentative structure of the compound FG 2 was assigned as below:



### PRESENCE OF PRENYL CHAIN:

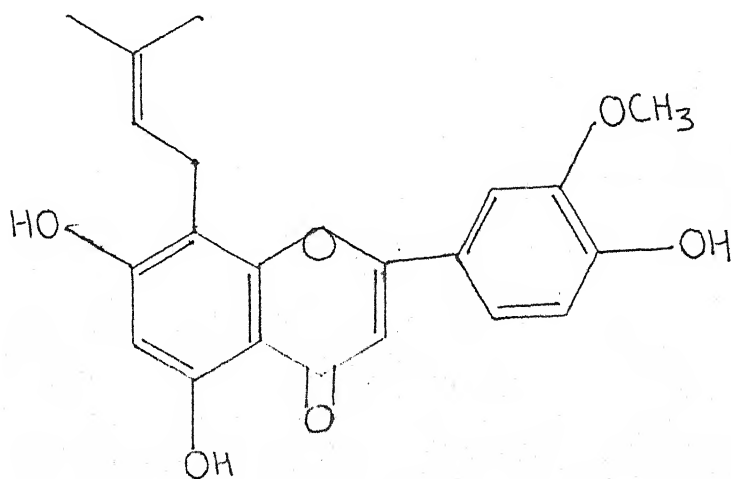
The IR spectrum of the compound FG 2, showed peaks at  $\nu_{\text{max}}^{\text{KBr}}$  1374 and  $1386\text{ cm}^{-1}$ , which were due to the presence of gem dimethyl group<sup>18</sup>.

In the electron impact mass spectrum of the compound FG 2, the fragment ion peaks at  $m/z$  312 and  $m/z$  313 were due to the loss of M-55 and M-56, which were indicating towards the prenylation adjacent to -OH group. This was further confirmed by the  $^1\text{H}$  NMR spectrum of the compound FG 2, which was almost similar to that of chrysoeriol with an exception due to the presence of 3, 3-dimethyl allyl group [ $\delta$  3.42, d, 2H, 6.3 Hz. H-1''; 5.21, t, 1H,  $J = 6.0$  Hz., H-2''; 1.66, s, 3H, H-4'' and 1.79, s, 3H, H-5'']<sup>19,20</sup>.

#### POSITION OF PRENYL SUBSTITUENT:

From the above discussion, it is evident that only position left for the attachment for the prenyl unit is at C-8. It was confirmed by the  $^1\text{H}$  NMR spectrum of the compound FG 2, which exhibited a singlet at  $\delta$  6.48 (1 H), assigned to H-6 of the ring A<sup>15</sup>. This proved the tri substituted nature of the ring A and confirmed the attachment of 3, 3- dimethyl allyl unit at C-8. The location of prenyl unit at C-8 was undoubtedly determined by the comparison of  $^{13}\text{C}$  NMR chemical shifts of C-6 and C-8 [97.1(d) & 105.3 (s) respectively] of the 6 or 8 substituted 5, 7- dihydroxy flavones<sup>21</sup>

Thus, in light of the above discussions, the following structure can be assigned to the aglycone FG 2

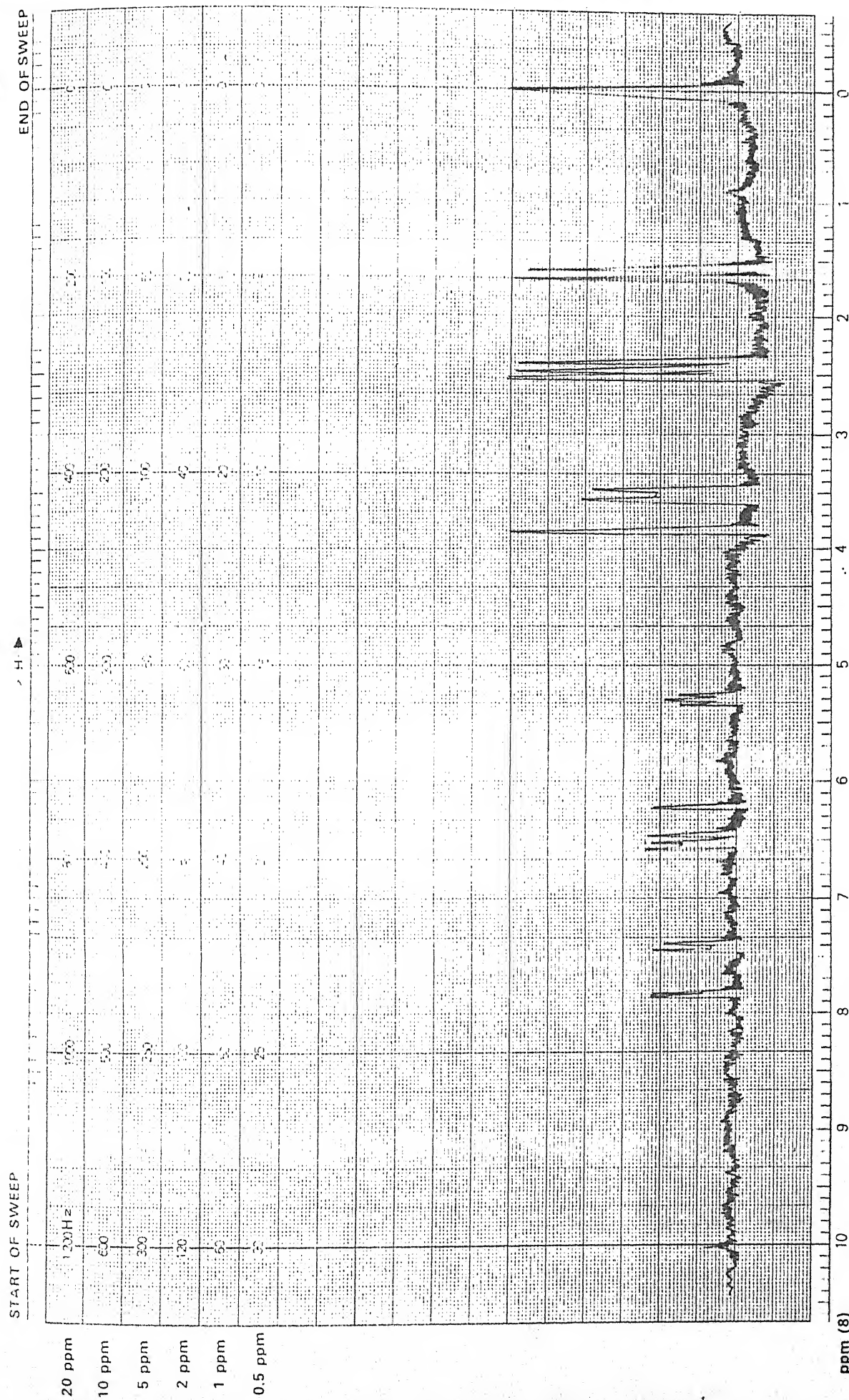


# <sup>1</sup>H NMR SPECTRUM OF THE TRI ACETYL DERIVATIVE OF THE AGLYCONE:

The above structure was found to be in full accordance with the <sup>1</sup>H NMR spectrum of the tri acetyl derivative of the aglycone FG 2. The important signals recorded in the <sup>1</sup>H NMR spectrum of the aglycone and the structural units inferred with the help of available literature<sup>9,15,22</sup> are given below in the Table 3 (Fig 3).

TABLE 3

S. NO.	δ	PATTERN	J VALUE	NO. OF	ASSIGNMENTS
	VALUE		IN HZ.	PROTONS	
1.	6.26	s	-	1	H-3
2.	6.48	s	-	1	H-6
3.	7.82	d	1.5	1	H-2'
4.	6.82	d	8.5	1	H-5'
5.	7.49	dd	1.5 & 8.5	1	H-6'
6.	3.42	d	6.3	2	H-1''
5.	5.21	t	6.0	1	H-2''
6.	1.66	s	-	3	H-4''
7.	1.79	s	-	3	H-5''
8.	3.88	s	-	3	3'-OCH <sub>3</sub>
9.	2.49	s	-	3	5-OAc
10.	2.42	s	-	3	7-OAc
11.	2.32	s	-	3	4'-OAc



LOCK POS. \_\_\_\_\_ ppm SPECTRUM AMPL. \_\_\_\_\_ SWEEP TIME \_\_\_\_\_ min NUCLEUS \_\_\_\_\_ SAMPLE \_\_\_\_\_ OPERATOR \_\_\_\_\_

LOCK POWER \_\_\_\_\_ mG FILTER \_\_\_\_\_ Sec SWEEP WIDTH \_\_\_\_\_ ppm ZERO REF. \_\_\_\_\_ DATE \_\_\_\_\_

DECOUPLE POS. \_\_\_\_\_ ppm RF POWER \_\_\_\_\_ mG END OF SWEEP \_\_\_\_\_ ppm SAMPLE TEMP. \_\_\_\_\_ °C SOLVENT \_\_\_\_\_ SPECTRUM NO \_\_\_\_\_

DECOUPLE POWER \_\_\_\_\_ mG

Fig. 3

### $^{13}\text{C}$ NMR SPECTRUM OF AGLYCONES FG 2:

In the  $^{13}\text{C}$  NMR spectrum of the compound FG 2, some important peaks were observed, which were helpful in the assignment of the C skeleton of the molecule. The  $^{13}\text{C}$  NMR spectral data and the positions assigned to the C atoms with the help of available literature<sup>15,22& 23</sup> are given in the following Table 4.

TABLE 4

S. NO.	$\delta$ VALUE	PATTERN	ASSIGNMENTS
1.	164.9	s	C-2
2.	103.7	d	C-3
3.	182.6	s	C-4
4.	159.7	s	C-5
5.	97.1	d	C-6
6.	158.4	s	C-7
7.	105.3	s	C-8
8.	156.8	s	C-9
9.	104.6	s	C-10
10.	121.2	s	C-1'
11.	112.4	d	C-2'
12.	148.4	s	C-3'
13.	153.3	s	C-4'
14.	112.9	d	C-5'

15.	119.8	d	C-6'
16.	61.7	q	-OCH <sub>3</sub>
17.	22.3	t	C-1''
18.	123.6	d	C-2''
19.	131.4	s	C-3''
20.	25.9	q	C-4''
21.	17.1	q	C-5''

### MASS SPECTRUM<sup>24</sup> OF THE AGLYCONE FG 2:

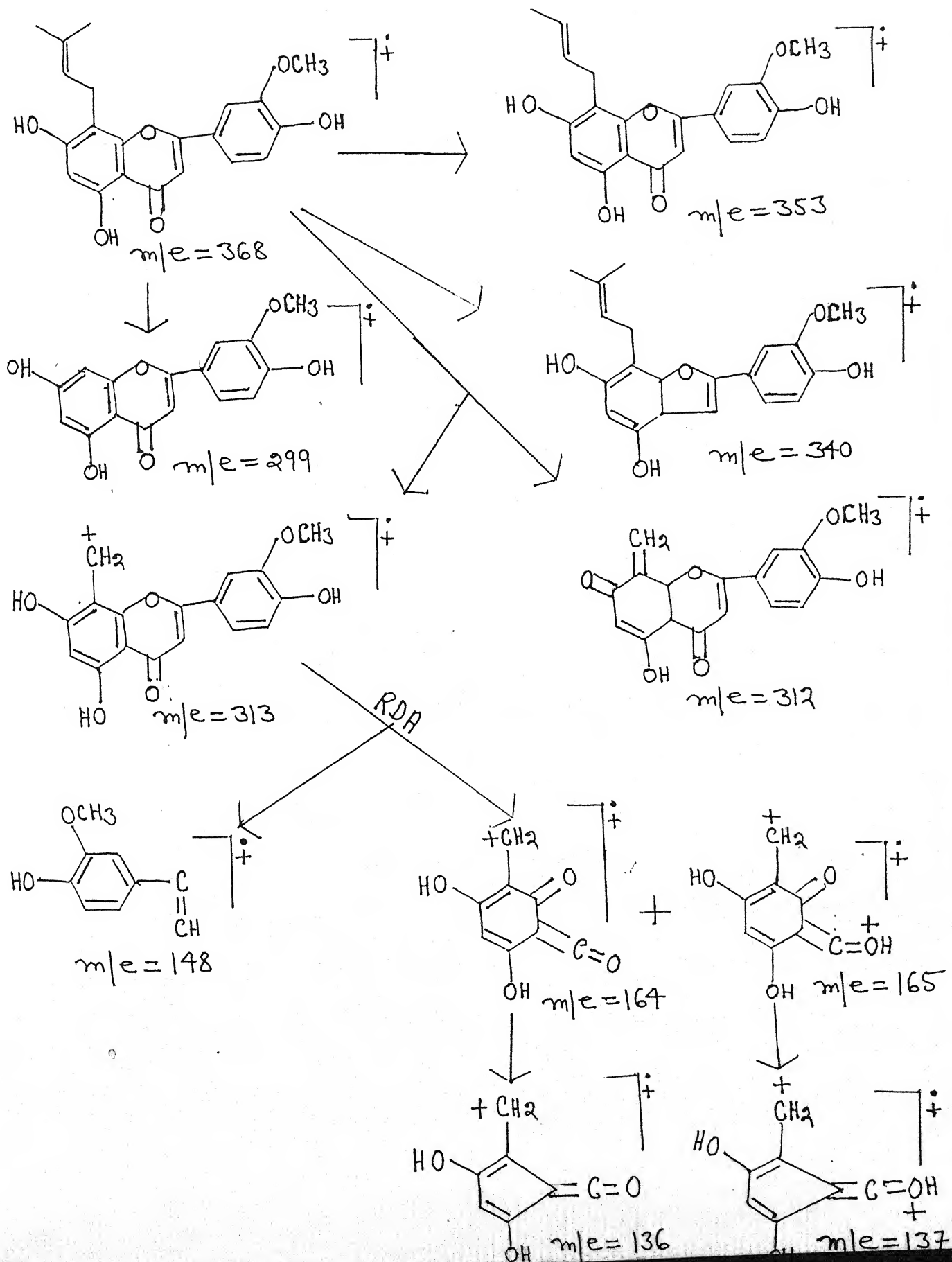
The structure assigned to the aglycone FG 2The mass spectra of the compound FG 2 was in full accordance with. The different species obtained during the fragmentation of the molecule are shown in the scheme I. The different m/z values obtained in the spectra are given below:

$M^+ = 368$  and  $m/z = 353, 340, 313, 312, 299, 165, 164, 148, 137$  and  $136$ .

### STUDY OF SUGAR:

After acid hydrolysis of flavonoid glycoside, the aq. hydrolysate obtained after separating the aglycone, was neutralised with barium carbonate and the barium sulphate formed was separated by filtration. The filtrate on concentration gave a yellowish viscous mass, which reduced Fehling's solution and gave colour with aniline hydrogen phthalate. This yellowish viscous mass was subjected to paper chromatography using aniline hydrogen phthalate as spraying reagent. As a result of this analysis, the presence of D- galactose, D- xylose and L- arabinose was established (confirmed by the Co-PC and Co-TLC with authentic samples).

# SCHEME-I



## **RATIO OF AGLYCON AND D-GALACTOSE IN THE GLYCOSIDE FG 1:**

The ratio of D-galactose, D-xylose and L-arabinose was found 1:1:1 by the study of  $^{13}\text{C}$  NMR and  $^1\text{H}$  NMR spectral data. Thus, by these spectral data, it was established that one molecule of glycoside was made of one molecule of aglycone and one molecule each of D-galactose, D-xylose and L-arabinose.

## **POSITION OF GLYCOSIDIC LINKAGE IN THE AGLYCON:**

The position of glycosidic linkage in aglycone was ascertained by the comparison of UV spectral data of aglycone with that of glycoside FG 1 and was assigned on position 4' in the B ring of the glycoside FG 1 on the basis of the following facts:

The sugars could be linked to the aglycone through the 5-OH or 7-OH positions in the ring A or through the 4' -OH group in the ring B (as it is a -O-glycoside evident from its acid hydrolysis).

The glycoside FG 1 when reacted with  $\text{FeCl}_3$ , gave an intense green colour pointing towards the presence of ortho hydroxy carbonyl grouping in the molecule of the glycoside due to a free 5 -OH group<sup>25</sup>. Further on addition of  $\text{AlCl}_3/\text{HCl}$ , in the UV spectrum of the glycoside FG 1, a +40 nm bathochromic shift appeared, which was due to the presence of a free 5 -OH group, thus, suggesting the presence of a free -OH group in the glycoside.

In the UV spectrum of the glycoside FG 1, a bathochromic shift at 29 nm was attributable to the presence of a free 7 -OH group. Further appearance of a new band at 320 nm, on addition of NaOMe was another evidence of the presence of a free 7 -OH group.

The aglycone FG 2, on addition of NaOMe showed a stable +56 nm bathochromic shift without decrease in intensity, which was due to the presence of a 4' - OH group in free state. This shift was absent in the glycoside, FG 1, which was a confirmation of the glycosidic linkage at this site. Thus, the sugars are attached to the aglycone in the trisaccharide form through the 4' position, as it is the only site available for the glycosylation. Therefore, all the sugars are attached to this position.

#### SEQUENCE OF SUGARS IN THE FLAVONOID GLYCOSIDE FG 1:

The compound FG 1 was hydrolysed with Killiani's mixture<sup>26</sup> at room temperature, which liberated first D-xylose, followed by L- arabinose and then D- galactose, therefore it proved that D-xylose was terminal sugar and D-galactose was linked to the aglycone. On column chromatography of contents over Si gel, using methanol as eluant, a mixture of three proaglycones was obtained.

1. FG-PA<sub>1</sub> [Molecular formula C<sub>27</sub>H<sub>30</sub>O<sub>11</sub>, M<sup>+</sup> 530 (EIMS)].
2. FG-PA<sub>2</sub> [Molecular formula C<sub>32</sub>H<sub>38</sub>O<sub>15</sub>, M<sup>+</sup> 662 (EIMS)]
3. FG-PA<sub>3</sub> [Molecular formula C<sub>37</sub>H<sub>46</sub>O<sub>19</sub>, M<sup>+</sup> 794 (EIMS)].

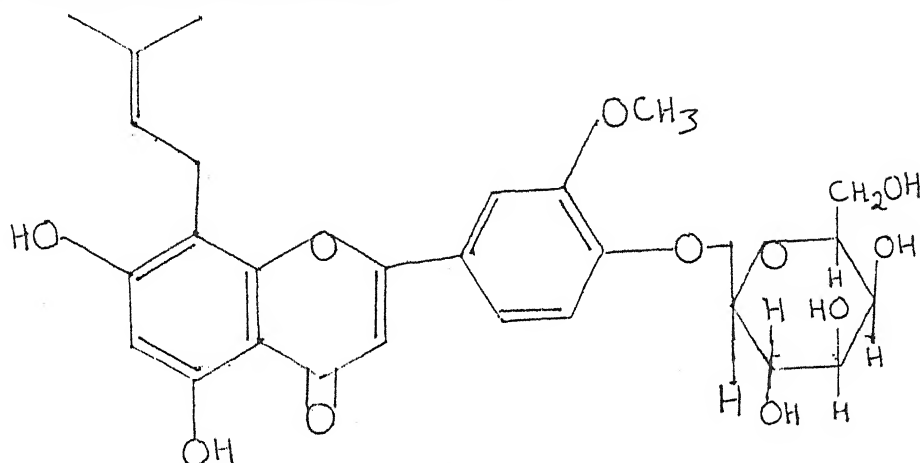
#### STUDY OF PROAGLYCONE FG -PA<sub>1</sub>:

The proaglycone (FG -PA<sub>1</sub>) was hydrolysed with 7% sulphuric acid, when it yielded aglycone and D-galactose (confirmed by Co-PC and Co TLC). The aglycone was identified by mmp, Co-PC and Co-TLC.

This proaglycone was hydrolysed by enzyme emulsin to yield D- galactose and aglycone, therefore, the D-galactose was linked to the aglycone by the  $\beta$  linkage.

### PERMETHYLATION AND HYDROLYSIS OF FG-PA<sub>1</sub>:

The proaglycone FG-PA<sub>1</sub> was permethylated by the method of Khun<sup>27</sup>, followed by hydrolysis, to give 2, 3, 4, 6-tetra-O-methyl -D-galactose (identified by Co-PC and Co-TLC), thus, exhibiting that C-1 of the D- galactose was linked to the 4' of the aglycone. It also suggested that D-galactose was present in the pyranose form. Therefore, FG-PA<sub>1</sub> was assigned the structure as given below:



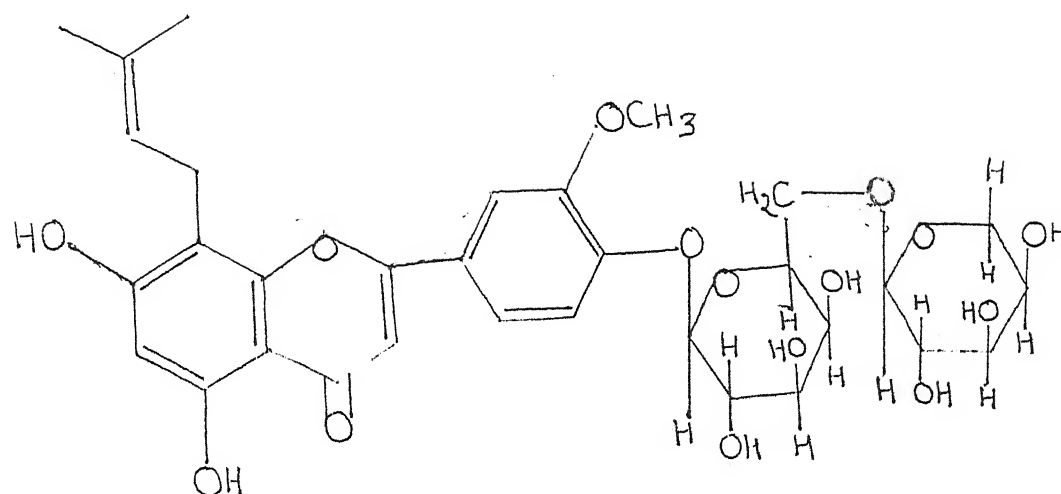
### STUDY OF THE PROAGLYCONE FG -PA<sub>2</sub>:

The FG-PA<sub>2</sub> on acid hydrolysis with 7% sulphuric acid yielded aglycone, D-galactose and L-arabinose (confirmed by the Co PC and Co TLC).

### PERMETHYLATION AND HYDROLYSIS OF FG-PA<sub>2</sub>:

The FG-PA<sub>2</sub> was permethylated by the Khun's procedure followed by hydrolysis to yield 2, 3, 4-tri-O-methyl-D-galactose and 2, 3, 4-tri-O-methyl-L-arabinose (identified by Co-PC and Co-TLC).

Thus, it suggested that L- arabinose was linked to the D-galactose by (1→ 6) linkage. It was also an indication of the fact that both the sugars were present in the pyranose form. Thus, FG -PA<sub>2</sub> was assigned the structure as given below:



### STUDY OF THE PROAGLYCONE FG-PA<sub>3</sub>:

FG-PA<sub>3</sub> on hydrolysing in the same manner as described for the FG-PA<sub>1</sub> and FG-PA<sub>2</sub>, yielded the aglycone FG 2 (confirmed by the mmp, Co-TLC and Co-PC) and sugars, D-galactose, L-arabinose and D-xylose (confirmed by the Co-PC and Co-TLC).

### PERMETHYLATION AND HYDROLYSIS OF THE FG-PA<sub>3</sub>:

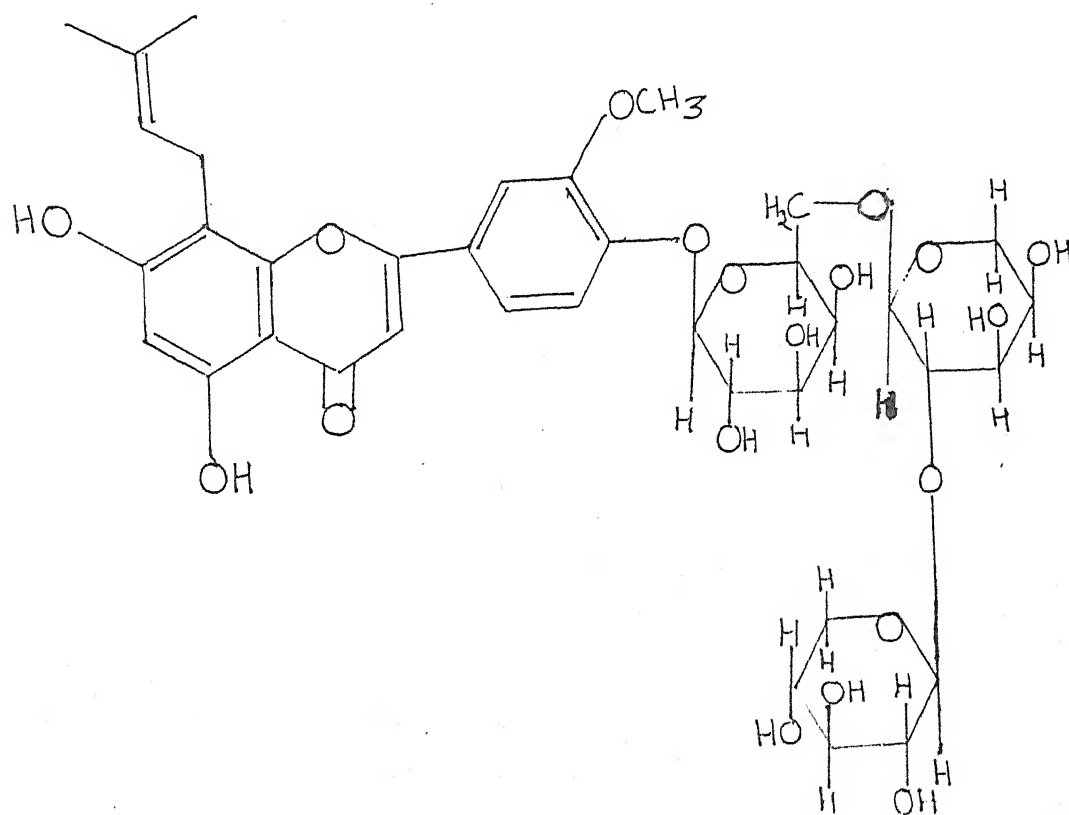
On permethylation followed by the hydrolysis, FG-PA<sub>3</sub> yielded 2, 3, 4-tri-O-methyl -D-galactose, 3,4-di-O-methyl-L-arabinose and 2, 3, 4-tri-O-methyl-D-xylose. Thus, clearly suggesting that D-xylose be linked to L-arabinose via. (1→2) linkage and indicated that these all sugars were present in the pyranose form.

### NATURE OF THE GLYCOSIDIC LINKAGE:

The glycoside FG 1 was first hydrolysed by enzyme almond emulsin<sup>28</sup>, when it yielded aglycone FG 2 (confirmed by mmp, Co-PC and Co-TLC), terminal sugar D-xylose and a disaccharide unit (identified by Co-PC and Co-TLC). This was a clear indication of  $\beta$  glycosidic linkage between D-xylose and disaccharide unit and also

between aglycone and disaccharide. The hydrolysed product having disaccharide unit was further hydrolysed by the enzyme takadiastase. It liberated the D-galactose and L-arabinose, thus, suggesting the presence of a  $\alpha$  linkage between L-arabinose and D-galactose.

Therefore, in light of the above discussed facts, the structure of the glycoside FG 1 can be given as 8-prenyl-chrysoeriol-4'-O- $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-galactopyranoside, which was assigned as follows:



The  $^1\text{H}$  NMR spectrum of its decaacetyl derivative and the  $^{13}\text{C}$  NMR and mass spectral data of the compound further supported this structure of the compound FG 1.

# $^1\text{H}$ NMR SPECTRUM OF DECA ACETATE OF GLYCOSIDE FG 1:

The chemical shifts obtained in the  $^1\text{H}$  NMR spectrum and the structural units inferred with the help of literature<sup>29,30</sup> are given below in the Table 5 (Fig 4).

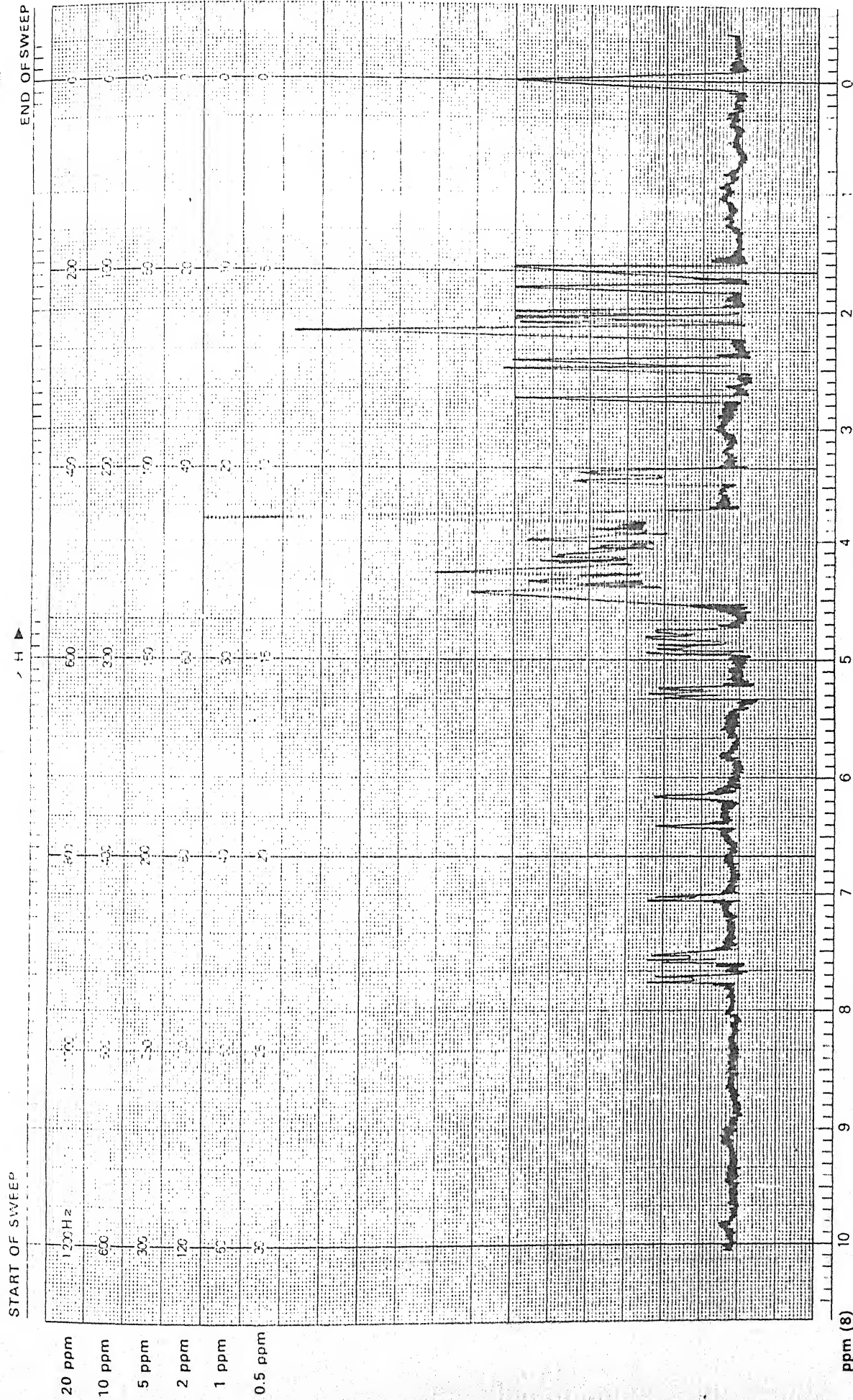
TABLE 5

S. NO.	$\delta$ VALUE	NO. OF PROTONS	PATTERN	J VALUE (HIZ.)	ASSIGNM- ENT
1.	6.23	1	s	-	H-3
2.	6.46	1	s	-	H-6
3.	7.85	1	d	1.5	H-2'
4.	7.03	1	d	8.5	H-5'
5.	7.51	1	dd	2.2 & 8.5	H-6'
6.	3.39	2	d	6.3	H-1''
7.	5.24	1	t	6.0	H-2''
8.	1.69	3	s	-	H-4''
9.	1.82	3	s	-	H-5''
10.	3.81	3	s	-	3' -OCH <sub>3</sub>
11.	2.47	3	s	-	5-OAc
12.	2.40	3	s	-	7-OAc

13.	4.74	1	d	7.2	1''' anomeric proton
14.	4.85	1	d	5.2	1'''' anomeric proton
15.	4.92	1	d	7.2	1''''' anomeric proton
16.	3.28-4.68	16	m	-	Protons of sugar
17.	2.07	3	s	-	2''' -OAc
18.	2.11	6	s	-	3''' & 4''' - OAc
19.	2.09	6	s	-	2'''' -OAc & 3'''' -OAc
20.	2.04	6	s	-	2''''' & 4''''' -OAc
21.	2.02	3	s	-	3''''' -OAc

### 13 C NMR SPECTRUM OF THE COMPOUND FG 1:

The signals obtained in the 13 C NMR spectrum of the compound FG 1 were utilised to interpret the C skeleton of the compound with the help of literature<sup>31,32</sup> and are given below in the Table 6.



OPERATOR

DATE

SPECTRUM NO

SAMPLE

NUCLEUS

SWEEP TIME

SPECTRUM AMPL.

ppm

LOCK POS.

ZERO REF.

SWEEP WIDTH

mG

LOCK POWER

SAMPLE TEMP. °C

SOLVENT

END OF SWEEP

mG

DECOUPLE POWER

Fig 11

TABLE 6

S. NO.	$\delta$ VALUE	PATTERN	ASSIGNMENT
1.	164.6	s	C-2
2.	103.9	s	C-3
3.	182.2	s	C-4
4.	159.3	s	C-5
5.	97.8	d	C-6
6.	157.7	s	C-7
7.	104.1	s	C-8
8.	157.2	s	C-9
9.	104.9	s	C-10
10.	124.9	s	C-1'
11.	111.7	d	C-2'
12.	150.6	s	C-3'
13.	151.4	s	C-4'
14.	115.2	d	C-5'
15.	120.7	d	C-6'
16.	61.1	s	3'-OCH <sub>3</sub>
17.	22.8	t	C-1''
18.	124.1	d	C-2''
19.	131.0	d	C-3''
20.	25.4	q	C-4''

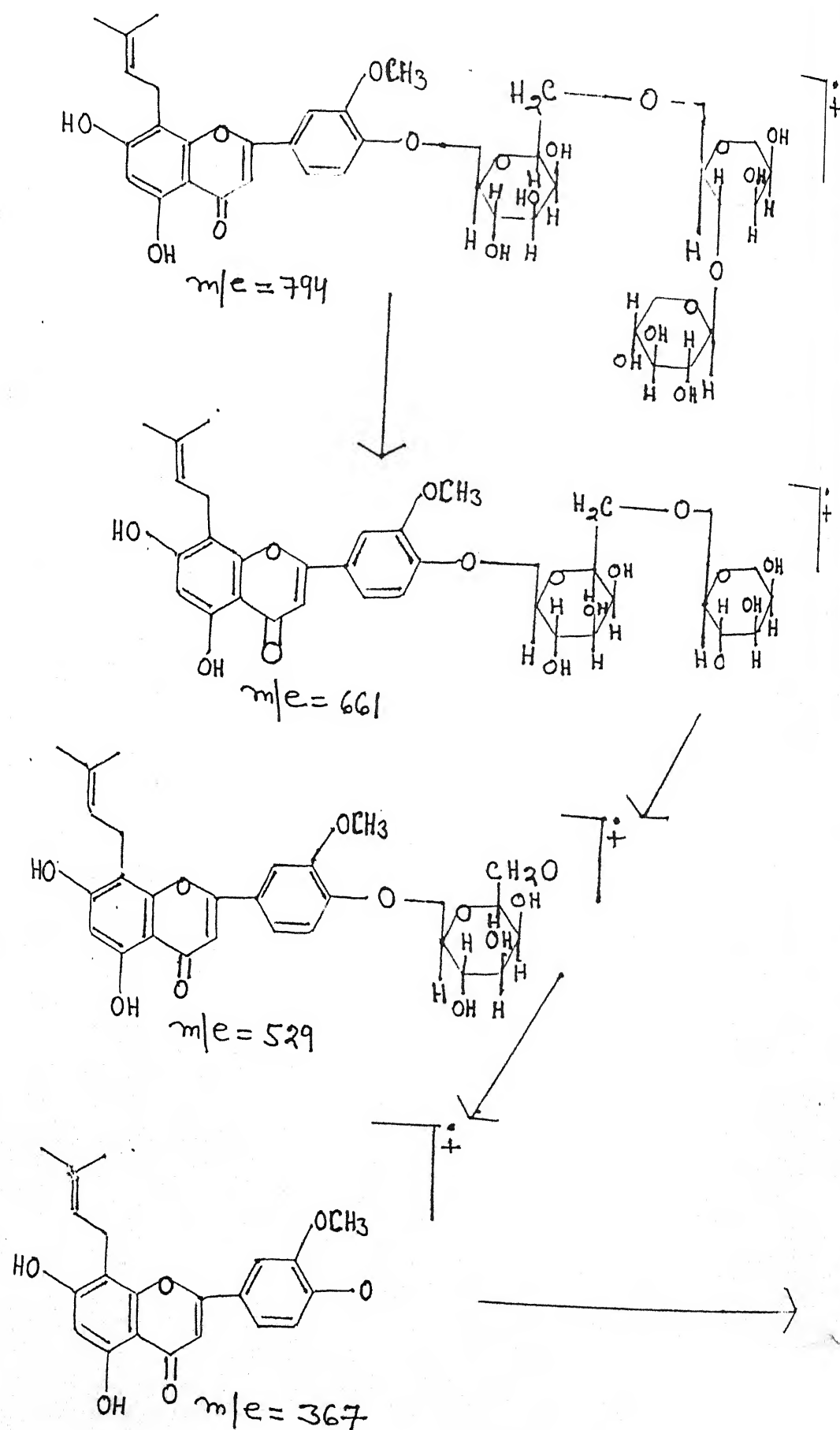
21.	17.8	q	C-5''
22.	103.2	d	C-1'''
23.	73.8	d	C-2'''
24.	76.4	d	C-3'''
25.	75.3	d	C-4'''
26.	76.5	d	C-5'''
27.	70.1	t	C-6'''
28.	101.6	d	C-1''''
29.	79.7	d	C-2''''
30.	73.0	d	C-3''''
31.	68.1	d	C-4''''
32.	63.3	t	C-5''''
33.	104.9	d	C-1'''''
34.	74.6	d	C-2'''''
35.	76.8	d	C-3'''''
36.	70.2	d	C-4'''''
37.	66.1	t	C-5'''''

#### MASS SPECTRUM<sup>33,34</sup> OF THE GLYCOSIDE FG 1:

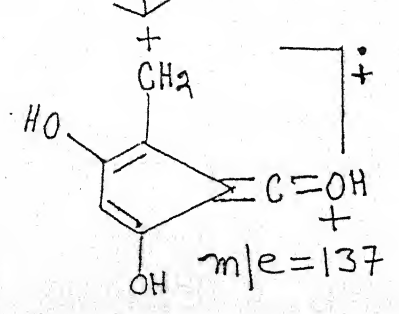
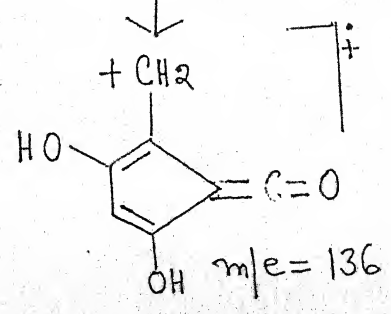
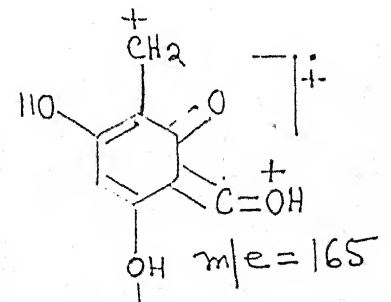
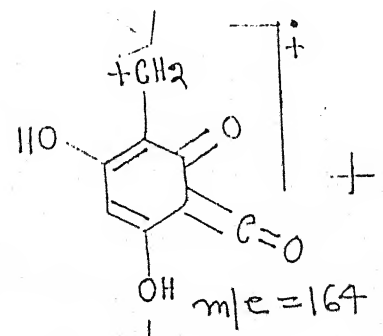
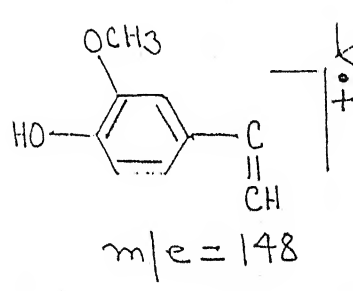
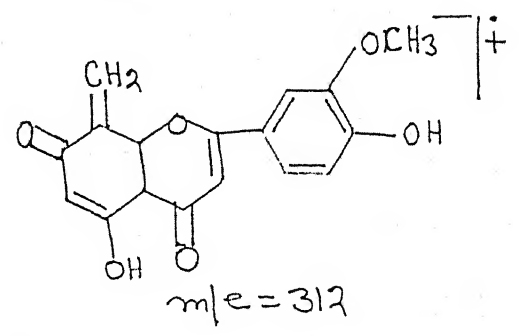
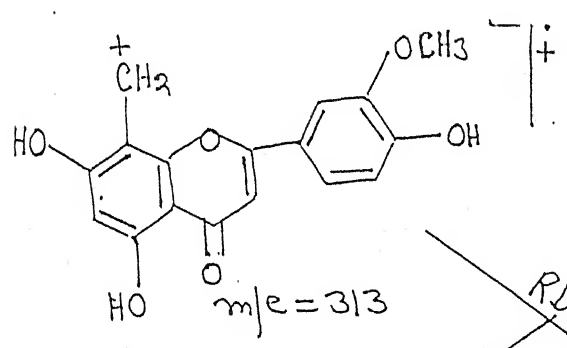
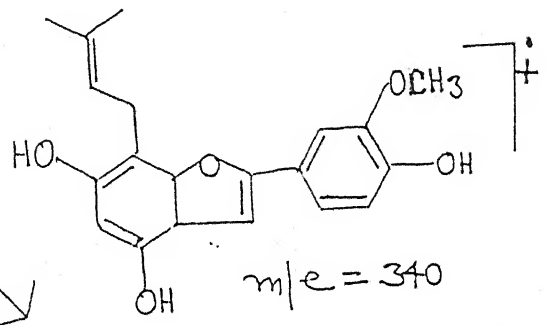
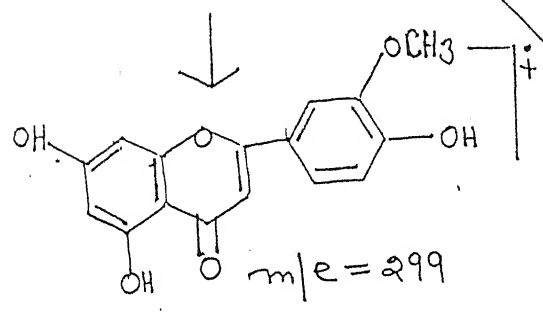
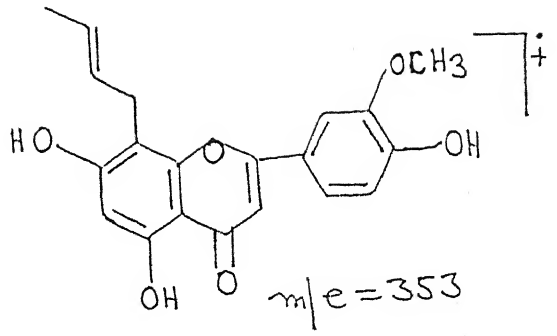
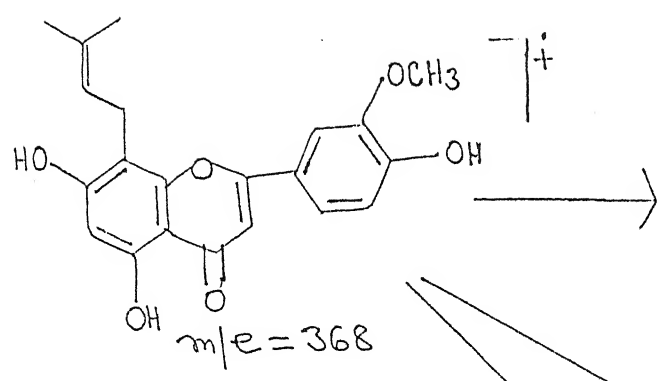
In the electron impact mass spectrum of the acylated glycoside FG 1, various fragment peaks were obtained, which are as given below:

$M^+$  794 and  $m/z$  661, 529, 368, 367, 353, 340, 313, 312, 299, 165, 164, 148, 137 and 136.

The scheme II shows various species assigned to fragments, which confirmed the proposed structure of the compound FG 1.



SCHEME-II



RDA

## EXPERIMENTAL

### EXTRACTION OF THE FLAVONOID GLYCOSIDE (FG):

The aerial parts of the *Kickxia ramosissima* (3.0 Kg.) were collected from the adjoining regions and were authenticated by the Botany Department of this college. The aerial parts were air dried, finely powdered and soxhlet extracted with 95% methanol. This methanol extract was concentrated and dissolved in cold water. The solution was then successively partitioned with n hexane, benzene, chloroform, ethyl acetate and methanol.

The n hexane, chloroform and methanol fractions were in negligible amount, when concentrated.

### STUDY OF ETHYL ACETATE SOLUBLE PART:

The ethyl acetate soluble part was concentrated under reduced pressure to get a brown viscous mass, which was subjected to column chromatography over a Si gel column. The column was eluted with  $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$  with increasing polarity. The  $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$  (4:6) part gave compound AFG 1, which was discussed in the Chapter 2 of this thesis. The  $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$  (1:9) part was homogeneous on TLC examination, thus, it was concentrated under reduced pressure and the residue was crystallised with methanol to get the compound FG 1 (1.85 g.).

### COLUMN CHROMATOGRAPHY:

Length of the column	90 cm.
Diameter of the column	5.0 cm.
Weight of the Silica gel	150 g.
Weight of the crude product	4.12 g.

TABLE 7

S. No.	Fraction no.	Eluant collected	Remarks
		50 ml. each	
1.	1-8	CHCl <sub>3</sub> : CH <sub>3</sub> OH 9:1	Sticky mass
2.	9-16	CHCl <sub>3</sub> : CH <sub>3</sub> OH 6:4	Not remarkable
3.	17-24	CHCl <sub>3</sub> : CH <sub>3</sub> OH 5:5	mixture
4.	25-32	CHCl <sub>3</sub> : CH <sub>3</sub> OH 4:6	Single spot (Ch. 2)
5.	33-40	CHCl <sub>3</sub> : CH <sub>3</sub> OH 3: 7	Mixture
6.	40-41	CHCl <sub>3</sub> : CH <sub>3</sub> OH 1:9	Single spot (FG 1)

#### STUDY OF THE COMPOUND FG 1:

The yellow coloured microcrystalline powdered compound FG 1, was soluble in methanol. It was analysed for molecular formula C<sub>37</sub>H<sub>46</sub>O<sub>19</sub>, m.p. 308-09°, molecular weight 794 (EIMS)

#### ELEMENTAL ANALYSIS:

Found	Calculated
C = 55.34%	55.92%
H = 6.16%	5.79%

Molecular weight = 794[EIMS]

Molecular formula = C<sub>37</sub>H<sub>46</sub>O<sub>19</sub>.

#### ACETYLATION OF THE COMPOUND FG 1:

Compound AFG 1 (100 mg.), fused sodium acetate (800 mg.) and the acetic anhydride (5 ml.) were taken in a flask fitted with air condenser, and heated on a

oil bath at 130° for 6 hours. The reaction mixture was poured in cold water, when a thick white ppt. was obtained, which was extracted with solvent ether. The ethereal layer was washed with water and sodium bicarbonate solution. Finally the ethereal layer was dried over anhydrous sodium sulphate and the ether was evaporated. The residue was crystallised from methanol as white needles (55 mg.), analysed for  $C_{57}H_{66}O_{29}$ ,  $M^+ 1214$

#### ELEMENTAL ANALYSIS:

Found	Calculated
C = 56.36%	56.34%
H = 5.59%	5.44%
Molecular weight = 1214 [EIMS]	Molecular formula = $C_{57}H_{66}O_{29}$
Acetyl group percentage = 34.94%	

#### . ACID HYDROLYSIS OF THE GLYCOSIDE FG 1:

The 800 mg. of compound FG 1 was refluxed with 100 ml. of 7% alcoholic  $H_2SO_4$  on a water bath for eight hours. After adding 50 ml. of water to the reaction mixture, the alcohol was removed by distilling under reduced pressure, when it yielded aglycone as a ppt. that was separated by filtration. The hydrolysate was neutralised with  $BaCO_3$  and was filtered to remove  $BaSO_4$ . The filtrate was concentrated under reduced pressure to a golden yellow mass that was examined by Co PC with authentic sample using n-Butanol: acetic acid: water (4:1:5) as a solvent system and aniline hydrogen phthalate as a spraying reagent.

## STUDY OF AGLYCONES FG 2:

The aglycone FG 2 was a yellowish microcrystalline powder (650 mg.) with m.p. 290-92° and analysed for molecular formula  $C_{21}H_{20}O_6$ ,  $M^+$  368. It responded positively to the tests of flavonoids.

### ELEMENTAL ANALYSIS OF COMPOUND FG 2:

Found	Calculated
C = 68.24%	68.48%
H = 5.81%	5.43%
Molecular weight = 368 [EIMS]	Molecular formula = $C_{21}H_{20}O_6$

### SCHINODA TEST:

Few crystals of the compound were dissolved in the 2 drops of EtOH and to this solution, Mg powder was added followed by the addition of 5M HCl. On viewing against a white background, red colour appeared.

### ACETYLATION OF THE AGLYCONES FG 2:

The compound FG was acetylated in the same manner as described for the FG 1.

### ELEMENTAL ANALYSIS:

Found	Calculated
C = 66.09%	65.59%
H = 5.33%	5.26%
Molecular weight = 494 [EIMS]	Molecular formula = $C_{27}H_{26}O_9$
Acetyl group % = 25.19%)	

## STUDY OF SUGAR HYDROLYSATE:

The sugar hydrolysate obtained after the acid hydrolysis was neutralised with barium carbonate and the ppt. of barium sulphate obtained was filtered off. The filtrate after concentration under vacuum was examined on PC, using aniline hydrogen phthalate as a spraying reagent. The inferences drawn are given in the Table 8.

TABLE 8

S. No.	Solvent system	Rf.value	Rf. value	Sugar identified
		Recorded	Found	
1.	Ethyl acetate :	0.38	0.37	D-xylose
	Water :	0.33	0.32	L-arabinose
	pyridine	0.235	0.22	D-galactose
	(2: 2: 1)			
2.	n-Butanol:	0.28	0.29	D- xylose
	Acetic acid:	0.21	0.20	L- arabinose
	Water	0.16	0.17	D- galactose
	(4: 1: 5)			

## PARTIAL HYDROLYSIS OF THE GLYCOSIDE FG 1:

The flavonoid glycoside FG 1 (400 mg.) and Killiani's mixture (70 ml. HCl : Acetic acid: Water, 15: 35: 50) were taken in a 150 ml. conical flask at room temperature and left for 4 days. The reaction mixture was extracted with n- butanol and the butanol extract on TLC (BAW) examination showed the presence of three compounds. This extract on concentration was subjected to the column chromatography

over a column of silica gel and eluting the column with chloroform: methanol in different proportions. This exercise resulted in the separation of three substances FG-PA<sub>1</sub>, FG-PA<sub>2</sub> and FG-PA<sub>3</sub>. The observations are recorded in the Table 9.

TABLE 9

1. Length of the column	90 cm.
2. Diameter of the column	3 cm.
3. Weight of the Si gel	100 g.
4. Weight of the butanol soluble part	380 mg.

S. No.	Fractions	Eluants	Spot on TLC plate	Substance
1.	1-8	Chloroform: methanol (4:1)	one	FG-PA <sub>1</sub>
2.	9-14	Chloroform: methanol (4:1)	two	FG-PA <sub>1</sub> + FG- PA <sub>2</sub>
3.	15-21	Chloroform: methanol (3:1)	one	FG-PA <sub>2</sub>
4.	22-27	Chloroform: methanol (3:1)	two	FG-PA <sub>2</sub> +FG- PA <sub>3</sub>
5.	28-35	Chloroform: methanol (1:1)	one	FG-PA <sub>3</sub>

### STUDY OF PROAGLYCONE (FG-PA<sub>1</sub>):

It analysed for the mol. for. C<sub>27</sub>H<sub>30</sub>O<sub>11</sub>, m.p. 341-42° and molecular weight M<sup>+</sup> 530 (EIMS).

#### ELEMENTAL ANALYSIS:

Found	Calculated
C = 61.36%	61.13%
H = 5.78%	5.67%
Molecular weight = 530 [EIMS]	Molecular formula = C <sub>27</sub> H <sub>30</sub> O <sub>11</sub>

#### PERMETHYLATION AND HYDROLYSIS:

The FG-PA<sub>1</sub> (50 mg.) was taken in a conical flask, with methyl iodide (5.0 ml.) and silver oxide in dimethyl formamide (8.0 ml.) and kept for two days at room temperature. The reaction mixture was filtered and residue was washed with dimethyl formamide. The filtrate was concentrated under reduced pressure to a syrupy mass. This mass was hydrolysed with 22% sulphuric acid, when it gave aglycone and methylated sugar. After separation of the aglycone, the aqueous part was neutralised with barium carbonate and barium sulphate formed was filtered off. The filtrate was concentrated and examined on paper chromatography using butanol: acetic acid: water (4: 1: 5) as solvent system and aniline hydrogen phthalate as spraying reagent. The sugar was identified as 2, 3, 4, 6-tetra-O-methyl-D-galactoside by Co-PC and Co-TLC.

#### STUDY OF PROAGLYCONE FG-PA<sub>2</sub>:

It analysed for the molecular formula C<sub>32</sub>H<sub>38</sub>O<sub>15</sub>, m.p. 324-25° and M<sup>+</sup> 662 (EIMS).

### ELEMENTAL ANALYSIS:

Found	Calculated
C = 58.56%	58.01%
H = 5.58%	5.74%
Molecular weight = 662 [EIMS]	Molecular formula = $C_{32}H_{38}O_{15}$

### PERMETHYLATION AND HYDROLYSIS:

The proaglycone FG-PA<sub>2</sub> was permethylated, followed by hydrolysis in the same manner as described for the proaglycone FG-PA<sub>1</sub>. It exhibited the presence of aglycone (identified by m.m.p., Co-TLC and Co-PC with authentic sample). The aq. hydrolysate revealed the presence of two methylated sugars identified as 2, 3, 4-tri-O-methyl-D-galactose and 2, 3, 4-O-methyl-L-arabinose (identified by Co-PC and Co-TLC).

### STUDY OF PROAGLYCONE FG-PA<sub>3</sub>:

It analysed for the molecular formula  $C_{37}H_{46}O_{19}$ , m.p. 308-09°, molecular weight 794 (EIMS)

### ELEMENTAL ANALYSIS:

Found	Calculated
C = 55.34%	55.92%
H = 6.16%	5.79%
Molecular weight = 794 [EIMS]	Molecular formula = $C_{37}H_{46}O_{19}$ .

### PERMETHYLATION AND HYDROLYSIS OF THE PROAGLYCONE FG-PA<sub>3</sub>:

Permethylation followed by hydrolysis was carried out in the similar manner as described for the FG-PA<sub>1</sub>. After the hydrolysis, three sugars, 2, 3, 4-tri-O-

methyl-D-galactose, 3, 4-di- O-methyl-L-arabinose and 2, 3, 4-tri-O-methyl-D-xylose were identified in the aq. hydrolysate. Presence of these methylated sugars further established the pyranose form of all the three sugars.

#### ENZYMATIC HYDROLYSIS:

The solution of flavonoid glycoside FG-1 (50 mg.) in ethanol (30 ml.) was suspended in an almond emulsin solution (30 ml.) in a conical flask. This reaction mixture was kept for three days at room temperature. The aglycone and hydrolysate were examined separately. The hydrolysate was subjected to paper chromatography with authentic sample using BAW (4:1:5) as solvent system and aniline hydrogen phthalate as spraying reagent. When it clearly revealed the presence of D-xylose and a disaccharide sugar unit.

Then the takadiastase enzyme was added in the reaction mixture. The mixture was again kept for three days. The ppt. was separated by filtration and hydrolysate was subjected to PC with authentic sample, when it showed three spots and revealed clearly the presence of D-galactose, D-xylose and L-arabinose (confirmed by Co-PC and Co-TLC with authentic samples).

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## CHAPTER IV

ISOLATION AND IDENTIFICATION OF A TRITERPENOIDAL SAPONIN  
GLYCOSIDE: "ECHINOCYSTIC ACID -3-O- $\beta$ -D-GALACTOPYRANOSIDE"  
FROM THE STEMS OF *ZIZIPHUS NUMMULARIA* (FAM. RHAMNACEAE)

*Ziziphus nummularia* belongs to the family Rhamnaceae and found in the arid and semiarid regions of Baluchistan, Cutch, Gujarat, Kathiawar, Punjab, Sind, S. Maharashtra and western Rajasthan (where it forms 14% of the total composition of grassland flora) and in the peninsular India, from Konkan to deccan and southwards and ascending to an altitude of 900 m.<sup>1, 2</sup>

*Z. nummularia* is commonly known as *Jhadiaber* or *Jharber* in Hindi and is a thorny, small bush or a shrub with widely divericating flexuous branches; stems and branches pale purplish or grey, velvety; stipular prickles in pairs; leaves ovate to orbicular, densely tomentose, beneath, serrate; flowers pale yellow, in axillary cymes; drupes red or black, 1 cm long<sup>1</sup>.

Animals graze the *Z. nummularia*, in its young and tender stage but after its development into hard, woody and thorny plant, only sheep and goats are able to browse on it. The leaves of this plant are rich in crude proteins and minerals. These leaves increase the nutritional status and carrying capacity of otherwise depleted and deficient grasslands and they are available even when other grasses have dried as these leaves shed in winter season after the appearance of red coloured berries and are collected, dried and stored to be used as a fodder for cattle, camels, sheep and goats. Fruits are not commercially very important but are edible and have pleasant sub acidic taste and are eaten and sold to a limited extent.

As far as medicinal properties are concerned the bark, leaves and fruits of this genus are used as folk medicines in the tropical and semitropical countries. The leaves of *Z. nummularia* are applied to scabies and boils<sup>2</sup> and the dried leaves are burnt to

inhale the smoke, which is useful in the treatment of coughs and cold.<sup>3</sup> The fruits are cooling and astringent and are also used in the bilious affections<sup>2</sup>.

The genus *Ziziphus* of the family rhamnaceae contains numerous species viz. *Z. fructus*, *Z. lotus*, *Z. mauritiana*, *Z. mucronata*, *Z. nummularia*, *Z. oenoplea*, *Z. rugosa*, *Z. spina cristi*, *Z. vulgaris* and *Z. xylopyrus*. Among these species *Z. mauritiana*, *Z. nummularia*, *Z. oenoplea* and *Z. xylopyrus* are commonly available in this region.

Many species of the genus *Ziziphus* are reported to contain cyclopeptide alkaloids, which are particularly common in the family rhamnaceae.<sup>4-11</sup> These alkaloids are polyamide plant bases containing a styrylamine unit as an integral part of 13, 14 or 15 membered macro cyclic ring.

The water insoluble fraction of the ethanol extract of the roots of *Z. jujube* afforded three new triterpene esters; 2-O-protocatechuoylaliphitalic acid, 2 $\alpha$ -hydroxypyracrenic acid and 3-O-protocatechuoylceanothic acid<sup>12</sup>. The bark of *Z. jujuba* afforded leucocynidine and the wood contained leucopelargonidin, betulinic acid and ceanothic acid.<sup>13</sup>

*Z. rugosa* afforded a triterpenoidal saponin named as rugoside, structure of which was given as jujubogenin -3-O- D - glucosyl - (1 $\rightarrow$  3) - L - arabinopyranoside by chemical and spectral methods.<sup>14</sup> An anti allergic compound ethyl -  $\alpha$  - D-fructofuranoside was separated from the ethyl alcohol extract of *Z. fructus*.<sup>15</sup>

The economic importance of family rhamnaceae is not very significant, thus, up to now, from a large number of *Ziziphus* sps, only *Z. jujuba* and *mauritiana* have been chemically investigated due to their cultivation as fruit trees. Although there is some

work done on the *Ziziphus nummularia* too. Thus, author decided to investigate this plant as it is a widely found local shrub, which is used for eating to a limited extent and possess some medicinal properties too. As far as work done on this plant is concerned there is a report of presence of tannins (14%) from the twig bark of *Z. nummularia*. The cyclopeptide alkaloids, which are very much common in the family rhamnaceae, were isolated from this plant also. The cyclopeptide alkaloids nummularine A, B and C; mucronine -D and amphibine-H were reported in the root bark of this plant.<sup>16</sup> In search of cyclopeptide alkaloids from the bark of *Z. nummularia*, the mauritine-A, scutianine -C, fragufofin and a 13 membered N-formyl cyclopeptide alkaloid, designated as nummularine- T were obtained.<sup>17-20</sup> The cyclopeptide alkaloids, nummularine -S and nummularine -C, too were isolated from the stem bark of the *Z. nummularia*.

#### ISOLATION OF THE TRITERPENOIDAL SAPONIN:

The stems of *Ziziphus nummularia* were air dried, powdered then extracted with 80% ethyl alcohol. The extract was concentrated under reduced pressure to get a dark brown viscous mass (850 mg.). This residue was partitioned between Et<sub>2</sub>O and water. The aq. layer was extracted with EtOAc followed by n- BuOH saturated with water. The n- BuOH fraction was subjected to chromatography on a silica gel column eluted with CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O in different ratios giving 46 fractions. Frs 21-36 were rechromatographed on a silica gel column eluted with CHCl<sub>3</sub>-EtOAc-MeOH (2:1:2). The solvent was evaporated and residue obtained was examined by TLC. Purification was done by dissolving it in MeOH with excess of ether and the process was repeated to find the colourless crystals of the compound TSG-1 on crystallisation with Me<sub>2</sub>CO. The saponin TSG-1 showed all the characteristic reactions of saponins.<sup>22-24</sup>

## STUDY OF THE TRITERPENOIDAL SAPONIN (TSG-1):

The compound TSG-1 was analysed for molecular formula  $C_{36}H_{58}O_9$ , m.p. 251-53° and  $M^+$  634 (EIMS). It showed the positive reactions of triterpenoidal saponins.<sup>25,26</sup>

### PRESENCE OF PENTACYCLIC TRITERPENOID:

The saponin was pentacyclic triterpenoid as it showed violet colour on its (0.5 ml. sol. in EtOH) reaction with 2,6-di tertiary -butyl-p-cresol (1.0 ml. sol. in ethanol<sup>27</sup>.

### UV SPECTRUM OF TSG-1:

In the UV spectrum of TSG-1 no absorption above 200 nm was observed.

### IR SPECTRUM OF TSG-1:

The IR spectrum of compound TSG-1 showed characteristic peaks, which were inferred for the structural identifications with the help of available literature.<sup>28-30</sup> These peaks and their structural assignments are given in Table 1 (Fig 1).

TABLE 1

S. No.	Wave Number $cm^{-1}$	Assignment
1.	3410	-OH group
2.	3030, 1625	C=C double bond
3.	2935	-CH stretching vibrations of methyl group
4.	1725	-COOH group
5.	1630	-CH <sub>2</sub> -CH

6.	1382, 1370	Gem dimethyl group
7.	1352, 1345	Triterpenoidal nature
8.	1057, 1104	Stretching of secondary alcoholic group
9.	810	CH=CH <sub>2</sub>

#### PRESENCE OF OH GROUP (S):

The IR spectrum of the compound showed a peak at  $\nu_{\text{max}}^{\text{KBr}}$  3410  $\text{cm}^{-1}$  which suggested the presence of OH group(s) in it.

#### NUMBER OF OH GROUP (S):

On acetylation of methyl ester (TSG 1e) of TSG-1 with acetic anhydride/pyridine, an acetylated product (TSG-1ac); mol. for.  $\text{C}_{47}\text{H}_{70}\text{O}_{14}$ , m.p. 276-277°,  $M^+$  858 was obtained. By the method of Weisenberger<sup>31</sup> the presence of five OH groups (% of acetyl group-24.91%) were established.

#### PRESENCE OF METHYL GROUP (S):

The IR peak at  $\nu_{\text{max}}^{\text{KBr}}$  2935  $\text{cm}^{-1}$  in compound TSG-1 suggested the presence of methyl group(s).

#### PRESENCE OF DOUBLE BOND:

Saponin TSG-1 gave positive colour test with tetranitro methane<sup>32</sup>, which showed the presence of double bond in it that was further confirmed by the appearance of absorption bands at  $\nu_{\text{max}}^{\text{KBr}}$  1625 and 3030  $\text{cm}^{-1}$  in the IR spectra of the compound.

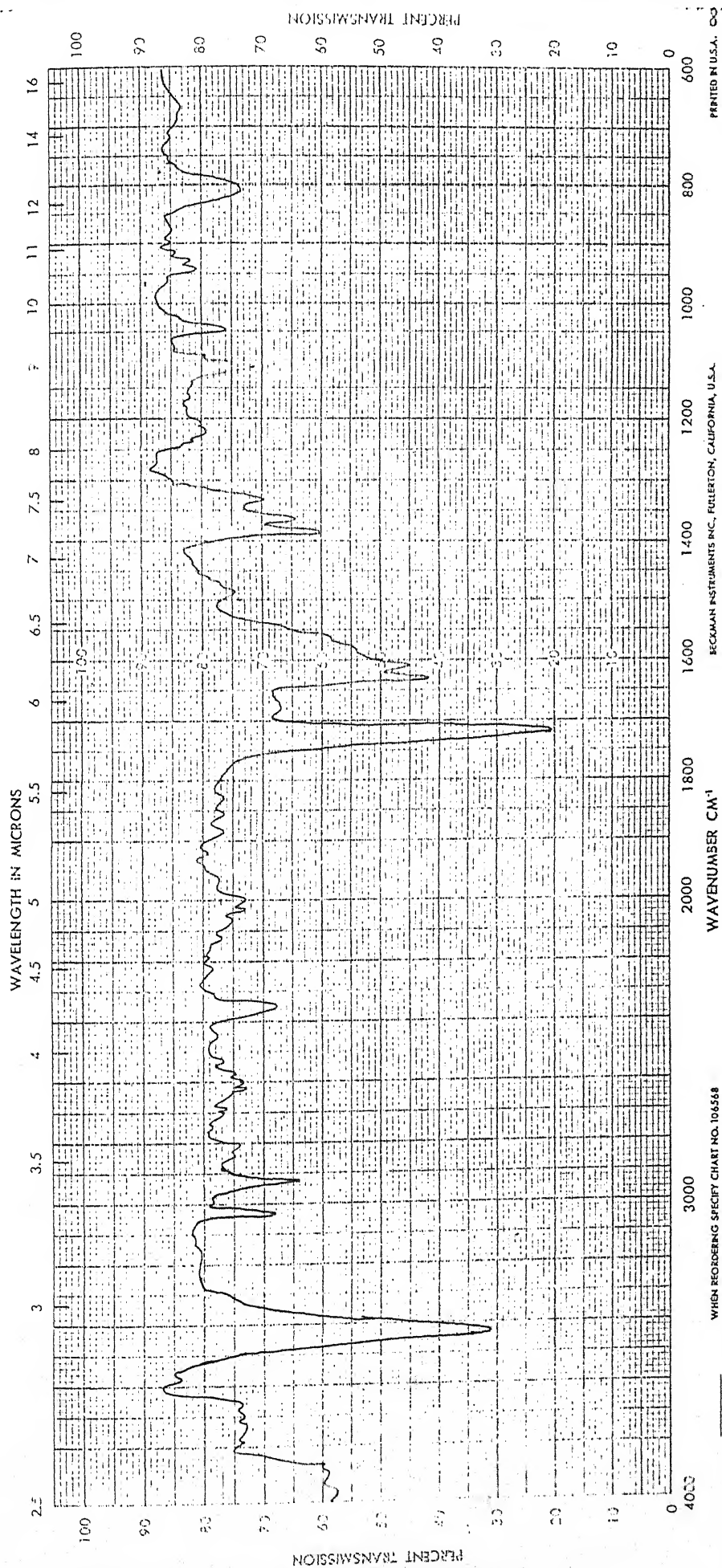


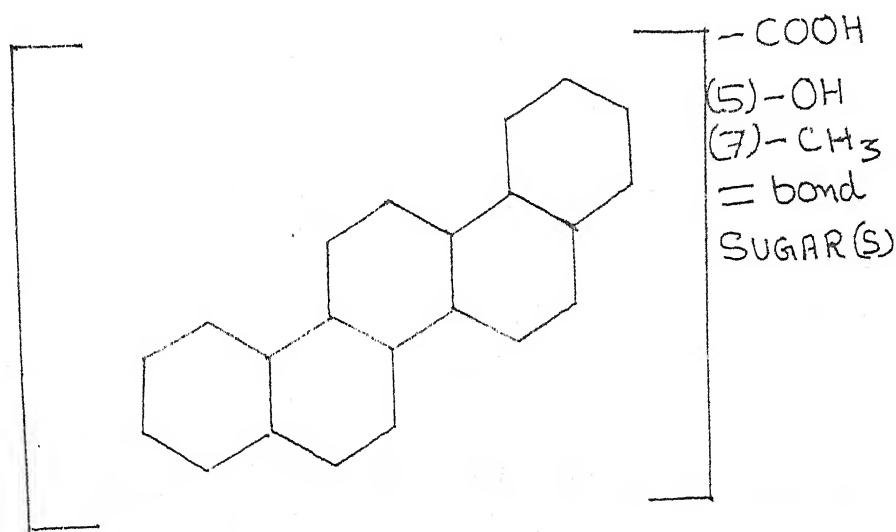
Fig. 1

### PRESENCE OF -COOH GROUP:

On treatment with  $\text{CH}_2\text{N}_2$ , compound TSG-1 gave methylated ester TSG 1c; molecular formula  $\text{C}_{37}\text{H}_{60}\text{O}_9$ , m.p.  $265-266^\circ$ ,  $M^+648$  (EIMS), indicating the presence of -COOH group which was further confirmed by the IR absorption peak at  $\nu_{\text{max}}^{\text{KBr}} 1725 \text{ cm}^{-1}$ .

### PRESENCE OF SUGAR UNIT (S):

Molisch's test<sup>33</sup> was shown positively by the compound TSG-1 that suggested the presence of sugar moiety(s) attached with the compound. Because of above data, the tentative structure of compound TSG-1 can be assigned as below:



### HYDROLYSIS OF THE SAPONIN (TSG -1):

On hydrolysis with 2N  $\text{H}_2\text{SO}_4$ , it yielded sugar(s) and a sapogenin (TSG-2). The sapogenin TSG-2 was separated by filtration and was studied further to establish the structure of this compound.

## ANALYSIS OF COMPOUND TSG-2:

Compound TSG -2 was insoluble in benzene but soluble in methanol and ethanol and gave all the colour reactions of triterpenoids<sup>34,35</sup> It was analysed for molecular formula  $C_{30}H_{48}O_4$ , m.p.  $302^\circ$  and  $M^+$  472 (EIMS).

## UV SPECTRUM OF THE COMPOUND TSG-2:

The UV absorption in methanol was not beyond 205 nm.

## IR SPECTRUM OF THE COMPOUND TSG-2:

In the IR region compound showed some significant peaks, which helped in inferring some important structural units with the help of available literature.<sup>36,37</sup> These are given below in the Table 2 (Fig 2).

Table 2

Wave No. (cm <sup>-1</sup> )	Assignment
3402	-OH group
3018, 1622	Double bond
2855	-CH stretching of Me group
2830	-CH stretching
1710	-COOH group
1635	-CH <sub>2</sub> -CH
1425, 1112	-C-O stretching of sec. OH group
1405, 1380, 1312	Triterpenoidal nucleus
1384, 1373	Gem di methyl group

Therefore, the following structure of sapogenin TSG -2 can be assigned on the basis of above observations

#### PRESENCE OF -OH GROUP(S) :

An absorption peak at  $\nu_{\text{max}}^{\text{KBr}}$  3402 cm<sup>-1</sup> in the infra red spectra showed the presence of -OH groups in sapogenin TSG 2.

On acetylation with Ac<sub>2</sub>O/pyr., it yielded an acetylated product ( TSG 2ac) with molecular formula C<sub>35</sub>H<sub>54</sub>O<sub>6</sub>, m.p. 235°, M<sup>+</sup> 570 EIMS. Two acetyl groups

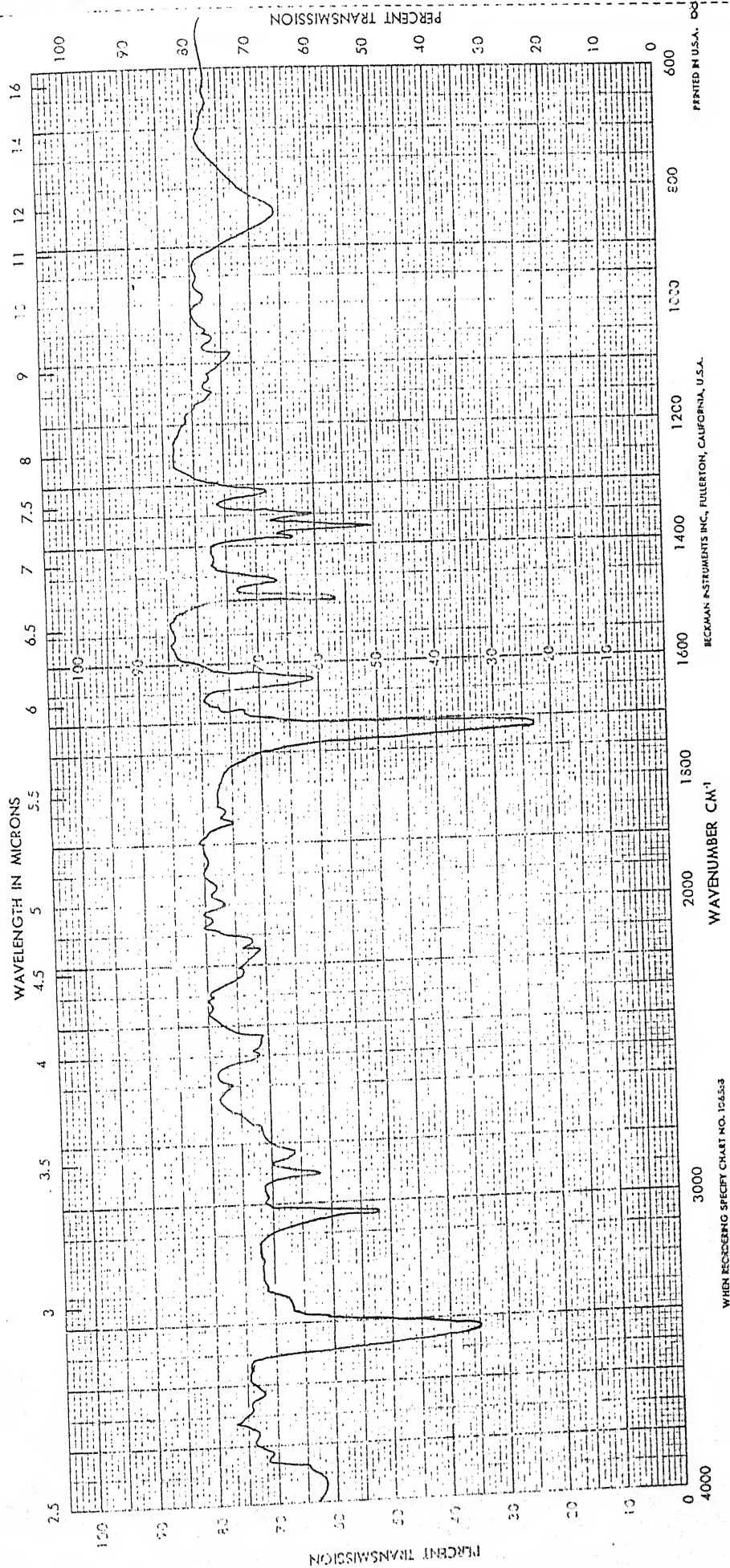


Fig. 2

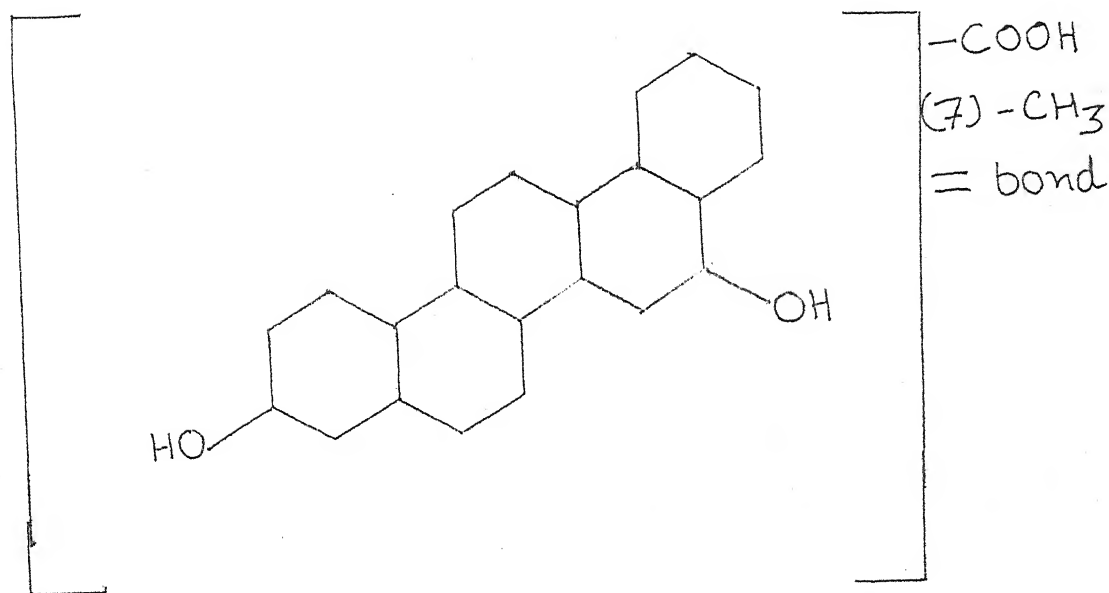
(14.93%) were estimated by using the Weisenberger process<sup>38</sup>, which confirmed the presence of two -OH groups in the compound TSG 2.

#### POSITION OF -OH GROUPS:

The <sup>1</sup>H NMR spectrum of the diacetyl derivative (TSG 2ac) indicated the position of -OH groups at C-3 and C-16 and their configuration as  $\beta$  and  $\alpha$  respectively due to the peaks at  $\delta$  3.05 (dd,  $J = 10.3, 9.0$  Hz, H-16 $\alpha$ ),  $\delta$  3.88 (dd,  $J = 11.4, 4.6$  Hz, H-3  $\beta$ )<sup>39</sup> and two singlets at  $\delta$  2.20 (3H, C-16  $\alpha$ , -OAc) and  $\delta$  2.08 (3H, C-3  $\beta$ , OAc). C-16 -OH group was assigned  $\alpha$  configuration due to downfield shift of Me-27 proton at  $\delta$  1.26 (s)<sup>39,40</sup> and C-3 -OH was assigned  $\beta$  configuration because of its large coupling constant (dd,  $J = 11.4, 4.6$  Hz.).<sup>39,41</sup>

On chromic acid oxidation<sup>42</sup> monomethyl ester of compound TSG 2 gave an oxidation product (TSG 2op); molecular formula C<sub>31</sub>H<sub>46</sub>O<sub>4</sub>, m.p. 207°, M<sup>+</sup> 482 (EIMS). This oxidation product on reacting with 2,4- dinitro phenyl hydrazine yielded dihydrazone derivative, confirming the oxidation product to be diketo derivative and thus, pointing towards the secondary nature of both the -OH groups of the sapogenin TSG 2. [See scheme III]. Oxidation product TSG 2op, on Zimmermann test<sup>43</sup> (an specific test for C-3 keto group) gave positive results, thus, establishing the position of one keto group at C-3 position, while, another group must be present at C-16, which was formed by the oxidation of secondary -OH group by chromic acid oxidation as stated above. Therefore, the positions of -OH groups were assigned at C-3 and C-16 in the compound TSG 2.

A tentative structure of compound TSG 2 is given on the basis of positions of OH groups as below:



#### PRESENCE OF METHYL GROUP(s):

A peak at  $\nu_{\text{max}}^{\text{KBr}}$  2855, 1373  $\text{cm}^{-1}$  in the IR spectrum of compound TSG 2 indicated the presence of methyl group(s).

#### POSITION OF METHYL GROUPS:

In the IR spectrum of the compound TSG 2, the peaks at 1373, 1384  $\text{cm}^{-1}$  showed the presence of gem dimethyl group. In the  $^1\text{H}$  NMR spectrum of acetylated derivative (TSG 2ac) peaks at  $\delta$  0.80, 0.84, 0.87, 0.91, 0.93, 1.01 and 1.26 (each 3H, s) were similar to the oleanane type of skeleton.<sup>44,45</sup> These peaks indicated the presence of seven tertiary methyl groups at C<sub>23</sub>, C<sub>24</sub>, C<sub>25</sub>, C<sub>26</sub>, C<sub>27</sub>, C<sub>29</sub> and C<sub>30</sub> positions.

#### PRESENCE OF DOUBLE BOND:

An absorption band at  $\nu_{\text{max}}^{\text{KBr}}$  3018, 1622  $\text{cm}^{-1}$  in the IR spectrum of compound TSG 2 indicated the presence of double bond. This was confirmed by the yellow coloration with TNM, an indication of double bond in one of the ring.

### POSITION OF DOUBLE BOND:

The position of double bond was established by the  $^1\text{H}$  NMR spectrum of sapogenin TSG 2. An upfield shift at  $\delta$  5.32 for vinyl proton H-12 and a downfield shift at  $\delta$  2.21 for H-11 and a peak at  $\delta$  2.18 for H-18 were a clear indication of the presence of double bond at 12-13. This was further suggested by the high terminal absorption at 188 nm in the UV spectrum<sup>46,47</sup> that is a characteristic of 12-13 double bond in oleanane series of majority of triterpenes. In the  $^{13}\text{C}$  NMR spectral data, the signals at 124 and 143.7 were attributable to the C-12 and C-13 confirming the double bond at this position<sup>40</sup>.

### PRESENCE OF $-\text{COOH}$ GROUP:

In the IR spectrum, a peak at  $\nu_{\text{max}}^{\text{KBr}}$  1710  $\text{cm}^{-1}$  indicated the presence of  $-\text{COOH}$  group. This was further corroborated by the fact that TSG 2 produced effervescence on treatment with sodium bicarbonate solution.

On treatment with  $\text{CH}_2\text{N}_2$ , compound TSG 2 produced a methyl ester TSG 2e (analysed for molecular formula  $\text{C}_{31}\text{H}_{50}\text{O}_4$ , m.p.  $221^\circ$  and  $\text{M}^+$  486(EIMS), thus, confirming the presence of acid group.

### POSITION OF $-\text{COOH}$ GROUP:

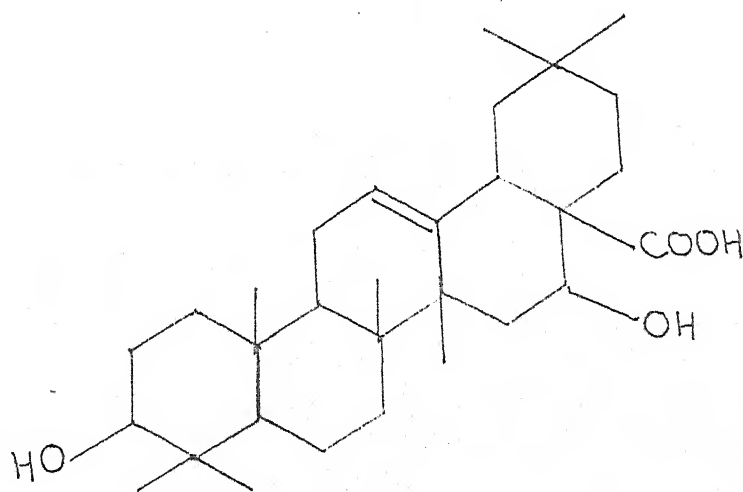
Sapogenin TSG 2 on heating gave a decarboxylated product, TSG 2d; molecular formula  $\text{C}_{29}\text{H}_{48}\text{O}_2$ , m.p.  $194^\circ$ ,  $\text{M}^+$  428 (EIMS). The  $^1\text{H}$  NMR spectrum of decarboxylated sapogenin TSG 2d, exhibited a signal at  $\delta$  2.63 (m, 1H) for H-17, whereas, no such peak for H-17 was observed in the spectra of mono methylated diacetyl derivative (TSG 2ac) of TSG 2, thus, corroborating the presence of  $-\text{COOH}$  group at C-

17 position. This position was further confirmed by the emergence of a  $^1\text{H}$  NMR peak at  $\delta$  3.60 (s, 3H) for  $-\text{COOMe}$  group<sup>47,48</sup> of its mono methylated diacetyl derivative. In the

In the mass spectra of compound fragment ions at 248 and 203 during Retro - Diels - Alder cleavage further suggested the position 17 of  $-\text{COOH}$  group<sup>49</sup>.

Saponification of mono methyl ester with 10% methanolic KOH underwent only partial saponification, however, when it was done with diethylene glycolic KOH, it again produced the sapogenin TSG 2. These results were a good confirmation of the fact that  $-\text{COOH}$  group was hindered and thus it must be at position C-17<sup>50</sup>.

In light of the above facts the structure of sapogenin TSG 2 was given as (I):  $3\beta$ ,  $16\alpha$ , olean-12-ene -28-oic acid, which was identified by the available literature<sup>51,52</sup> as a well known compound echinocystic acid.



This structure of compound TSG 2 was further corroborated by its  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and mass spectra.

# $^1\text{H}$ NMR SPECTRUM OF DIACETYLATED METHYL ESTER OF THE SAPOGENIN TSG 2:

The  $^1\text{H}$  NMR spectrum of diacetylated methyl ester of TSG 2 showed some interesting signals, which helped in inferring the structural assignments with the help of available literature<sup>53,54</sup>. These signals and their structural assignments are shown as below in Table 3 (Fig 3).

Table 3

S. No.	$\delta$ Value	No. of protons	Pattern	J Value in Hz	Structural Assignments
1.	0.97	3	s	-	C-23 Me group
2.	0.74	3	s	-	C-24 Me group
3.	0.91	3	s	-	C-25 Me group
4.	0.89	3	s	-	C-26 Me group
5.	1.26	3	s	-	C-27 Me group
6.	0.84	3	s	-	C-29 Me group
7.	0.96	3	s	-	C-30 Me group
8.	1.30-2.04	18	m	-	Polymethylene and methyl $\text{CH}_2$ and CH
9.	2.08	3	s	-	C-3-OAc
10.	2.18	1	s	-	H-18
11.	2.20	3	s	-	C-16-OAc

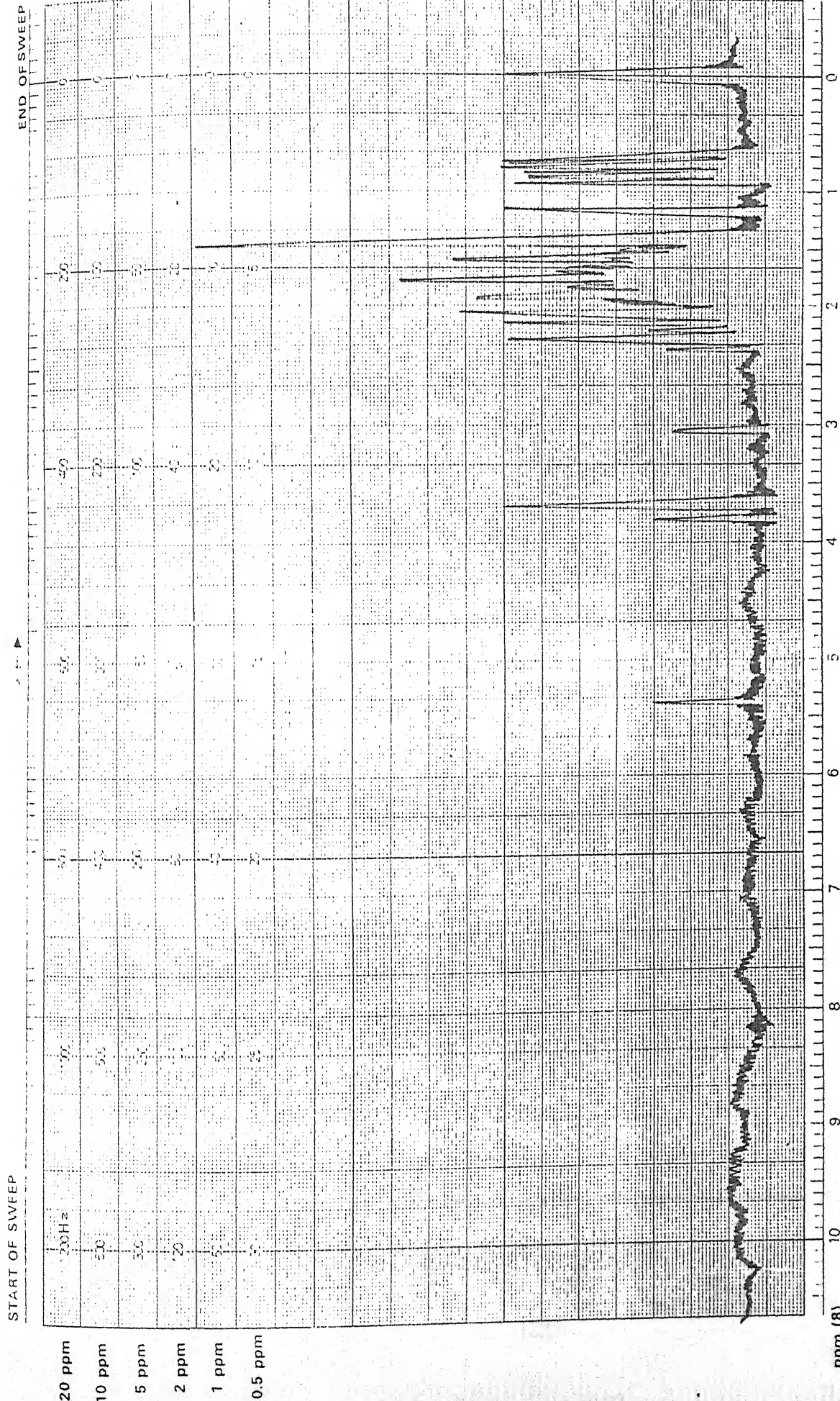
12.	2.21	1	s	-	H-11
13.	3.05	1	dd	10.3, 9.0	H-16 $\alpha$
14.	3.60	3	s	-	COOMe
15.	3.88	1	dd	11.4, 4.6	H-3 $\beta$
16.	5.32	1	dd	6.9, 10.1	Vinylic proton at C-12

### <sup>13</sup>C NMR SPECTRUM OF THE COMPOUND TSG 2 :

Sapogenin TSG 2 showed important peaks in its <sup>13</sup>C NMR spectrum. With the help of available literature<sup>55, 56</sup> these peaks helped in structural assignments of TSG 2, which are given in Table 4:

Table 4

S. No.	$\delta$ Value	Assignment
1.	39.5	C-1
2.	28.6	C-2
3.	90.4	C-3
4.	39.4	C-4
5.	57.8	C-5
6.	17.1	C-6
7.	33.2	C-7
8.	40.7	C-8



START OF SWEEP \_\_\_\_\_ END OF SWEEP \_\_\_\_\_

LOCK POS. \_\_\_\_\_ ppm SPECTRUM AMPL. \_\_\_\_\_ SWEEP TIME \_\_\_\_\_ min NUCLEUS \_\_\_\_\_ SAMPLE \_\_\_\_\_ OPERATOR \_\_\_\_\_

LOCK POWER \_\_\_\_\_ mG FILTER \_\_\_\_\_ Sec ZERO REF. \_\_\_\_\_ DATE \_\_\_\_\_

DECOUPLE POS. \_\_\_\_\_ ppm RF POWER \_\_\_\_\_ mG END OF SWEEP \_\_\_\_\_ ppm SAMPLE TEMP. \_\_\_\_\_ °C SOLVENT \_\_\_\_\_ SPECTRUM NO. \_\_\_\_\_

DECOUPLE POWER \_\_\_\_\_ mG

Fig. 3

---

9.	48.4	C-9
10.	38.1	C-10
11.	25.4	C-11
12.	124.0	C-12
13.	143.7	C-13
14.	42.2	C-14
15.	28.9	C-15
16.	67.6	C-16
17.	46.8	C-17
18.	42.1	C-18
19.	47.1	C-19
20.	32.0	C-20
21.	35.1	C-21
22.	32.8	C-22
23.	28.6	C-23
24.	18.3	C-24
25.	15.4	C-25
26.	17.0	C-26
27.	26.0	C-27
28.	178.6	C-28
29.	34.1	C-29
30.	23.6	C-30

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## MASS SPECTRUM OF THE COMPOUND TSG 2:

The compound TSG 2 showed some important fragmentation patterns<sup>57</sup> that are as given below:

$M^+ = 472, 440, 427, 248, 207, 203, 190, 189, 175$  and  $133$   $m/z$ .

The different fragmentation patterns obtained are shown in scheme -1.

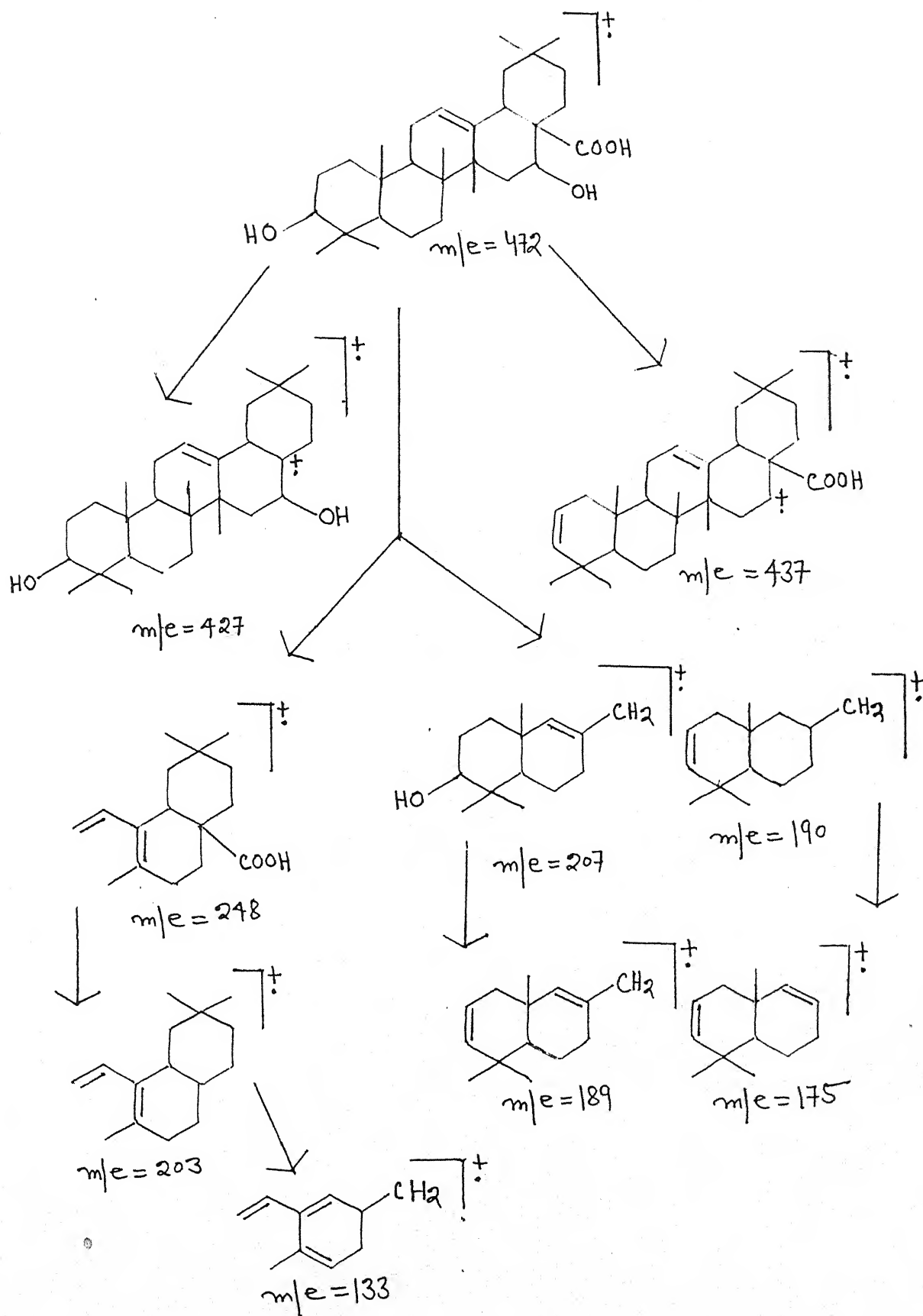
## STUDY OF THE SUGAR MOIETY (IES):

The compound TSG 1 on hydrolysis with  $2N$   $H_2SO_4$ , gave sapogenin TSG 2 and the aq. hydrolysate after filtration. The aq. hydrolysate was neutralised with  $BaCO_3$  and the  $BaSO_4$  obtained was removed by filtration. The filtrate after concentration reduced Fehlings solution, thus, suggesting the presence of sugar(s) attached with the sapogenin TSG 2.

The sugar was found to be D-galactose, when aq. hydrolysate was chromatographed on Whatmann No. 1 filter paper and aniline hydrogen phthalate was used as spraying agent. The Co PC and Co TLC with authentic sample<sup>58</sup> confirmed the presence of D-galactose (Rf.0.17) as only sugar moiety.

## THE PYRANOSE FORM OF THE D- GALACTOSE:

The D-galactose was present in the pyranose form, which was confirmed by the permethylation<sup>59</sup> followed by the acid hydrolysis of the saponin TSG 1. The presence of 2,3,4,6-tetra-O-methyl-D-galactose (authenticated by the Co PC and Co TLC) in the hydrolysate was a good confirmation of this fact. This also suggested that the C-1 position of the D-galactose was involved in the glycosidic linkage.



SCHEME-I

## NATURE OF THE GLYCOSIDIC LINKAGE:

The D-galactose was linked by the  $\beta$  linkage to the sapogenin TSG 2 which was proved by the enzymatic hydrolysis (almond -emulsin) and was further confirmed by the Co -PC and Co-TLC.

## POSITION OF GLYCOSIDIC LINKAGE IN COMPOUND TSG 2 :

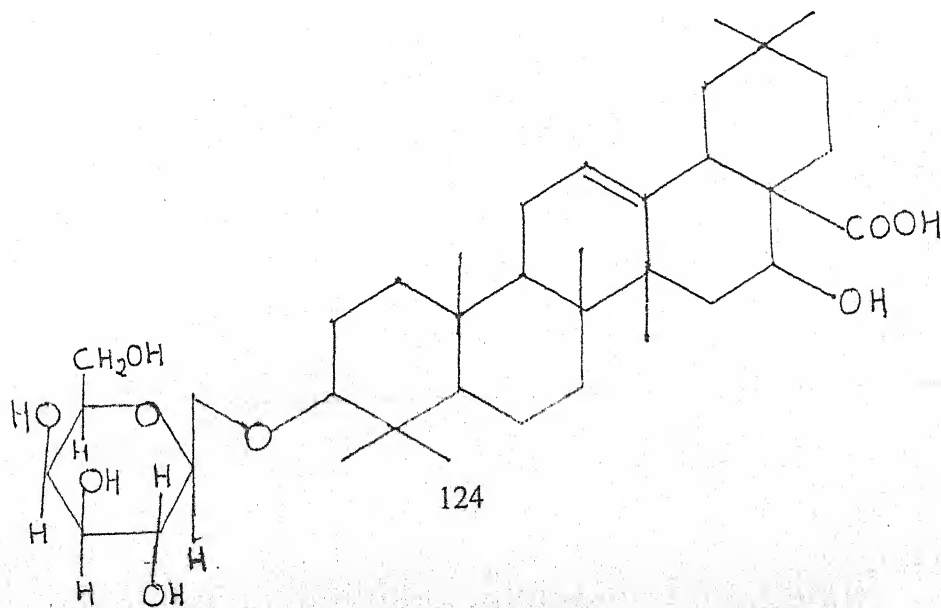
The glycosidic linkage in compound TSG 1 was possible in either of the three ways as described below:

- A. The galactose moiety could be linked through -COOH group at position C-17 or
- B. the linkage site could be through -OH group at C-17 or
- C. through -OH group at position C-3.

When the saponin was subjected to the alkaline hydrolysis, it did not produce any sugar and thus the possibility of linkage of galactose moiety through -COOH group was cancelled<sup>60</sup>. Thus, -COOH group was free in compound TSG 1.

The C-16 position of linkage was also not possible because of highly sterically hindered position of C<sub>16</sub>-OH group<sup>61</sup>. Therefore, lone possibility of linkage of D-galactose with sapogenin was through -OH at position C<sub>3</sub>.

Therefore, in light of these facts the structure of saponin TSG 1 can be assigned as below:



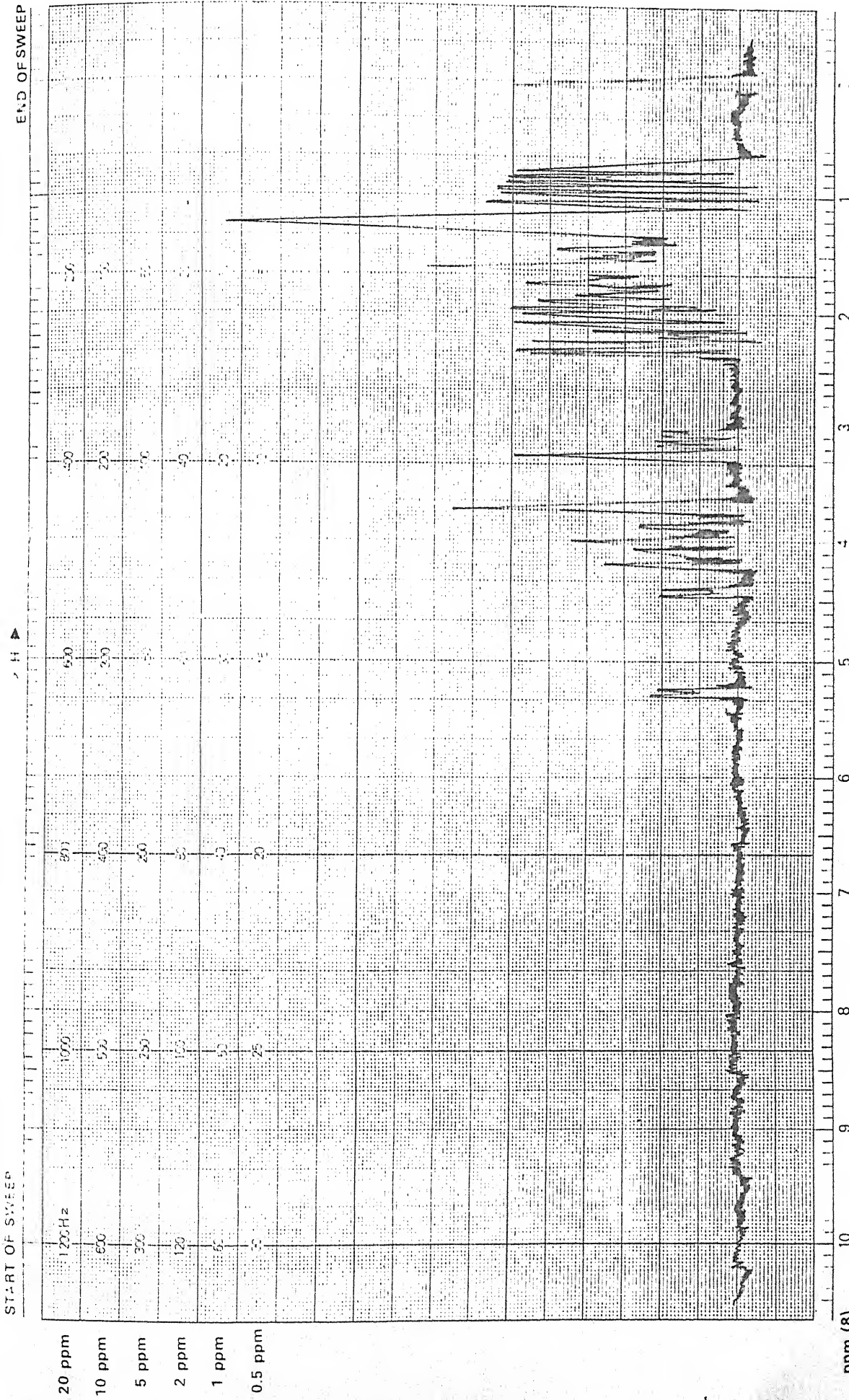
This structure of saponin TSG 1 was further confirmed by the  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and mass spectral data.

#### $^1\text{H}$ NMR SPECTRUM OF PENTA ACETYLATED DERIVATIVE (TSG 1ac):

The above structure was further confirmed by the  $^1\text{H}$  NMR of the tetra acetylated derivative of saponin TSG 1. The spectrum was interpreted with the help of available literature<sup>62</sup>. The important peaks and the structural units inferred are given in the Table 5 (Fig 4).

TABLE 5

S.NO.	$\delta$ Value	No. of Protons	Pattern.	J Value (Hz)	Assign- ments
1.	0.96	3	s	-	$\text{C}_{23}-\text{CH}_3$
2.	0.81	3	s	-	$\text{C}_{24}-\text{CH}_3$
3.	0.87	3	s	-	$\text{C}_{25}-\text{CH}_3$
4.	0.88	3	s	-	$\text{C}_{26}-\text{CH}_3$
5.	1.12	3	s	-	$\text{C}_{27}-\text{CH}_3$
6.	0.86	3	s	-	$\text{C}_{29}-\text{CH}_3$
7.	0.95	3	s	-	$\text{C}_{30}-\text{CH}_3$
8.	1.15-2.05	18	m	-	Polymethyle ne and methyl $\text{CH}_3$ and CH
9.	5.25	1	dd	4.9	12-13 double bond



LOCK POS. \_\_\_\_\_ ppm  
LOCK POWER \_\_\_\_\_ mG  
DECOUPLE POS. \_\_\_\_\_ ppm  
DECOUPLE POWER \_\_\_\_\_ mG

FILTER \_\_\_\_\_ SeO  
SWEEP WIDTH \_\_\_\_\_ ppm  
RF POWER \_\_\_\_\_ mG

SWEEP TIME \_\_\_\_\_ min  
NUCLEUS \_\_\_\_\_  
SAMPLE \_\_\_\_\_

OPERATOR \_\_\_\_\_  
DATE \_\_\_\_\_  
SPECTRUM NO. \_\_\_\_\_  
SAMPLE TEMP. \_\_\_\_\_ °C

Fig. 4

10.	3.12	1	dd	8.14, 8.0	C <sub>3</sub> -1H
11.	3.04	1	dd	10.25, 9.10	C16-1H
12.	3.22	3	s	-	-COOCH <sub>3</sub>
13.	2.06	3	s	-	16-OAc
14.	2.19	1	d	4.2	18 $\beta$ -1H
15.	2.16	2	dd	4.3	CH <sub>2</sub> -11
16.	3.66-4.26	6	m	-	Protons of sugar moiety
17.	4.46	1	d	7.0	1' Anomeric proton
18.	2.02	3	s	-	2' OAc
19.	2.01	3	s	-	3' OAc
20.	1.94	3	s	-	4' OAc
21.	1.98	3	s	-	6' OAc

### 13 NMR SPECTRUM OF SAPONIN TSG 1:

The 13 NMR spectra of saponin TSG 1 showed some useful signals, which, helped in interpreting all C containing structural units which are given below in Table 6.

TABLE 6

S. No.	$\delta$ Value	Carbon NO.
1.	40.1	C-1
2.	28.1	C-2
3.	90.5	C-3
4.	40.6	C-4
5.	57.1	C-5
6.	17.3	C-6
7.	33.4	C-7
8.	39.3	C-8
9.	48.9	C-9
10.	37.8	C-10
11.	24.7	C-11
12.	123.9	C-12
13.	145.3	C-13
14.	43.5	C-14
15.	28.9	C-15
16.	66.2	C-16
17.	48.1	C-17
18.	42.1	C-18
19.	47.0	C-19
20.	30.5	C-20
21.	35.6	C-21

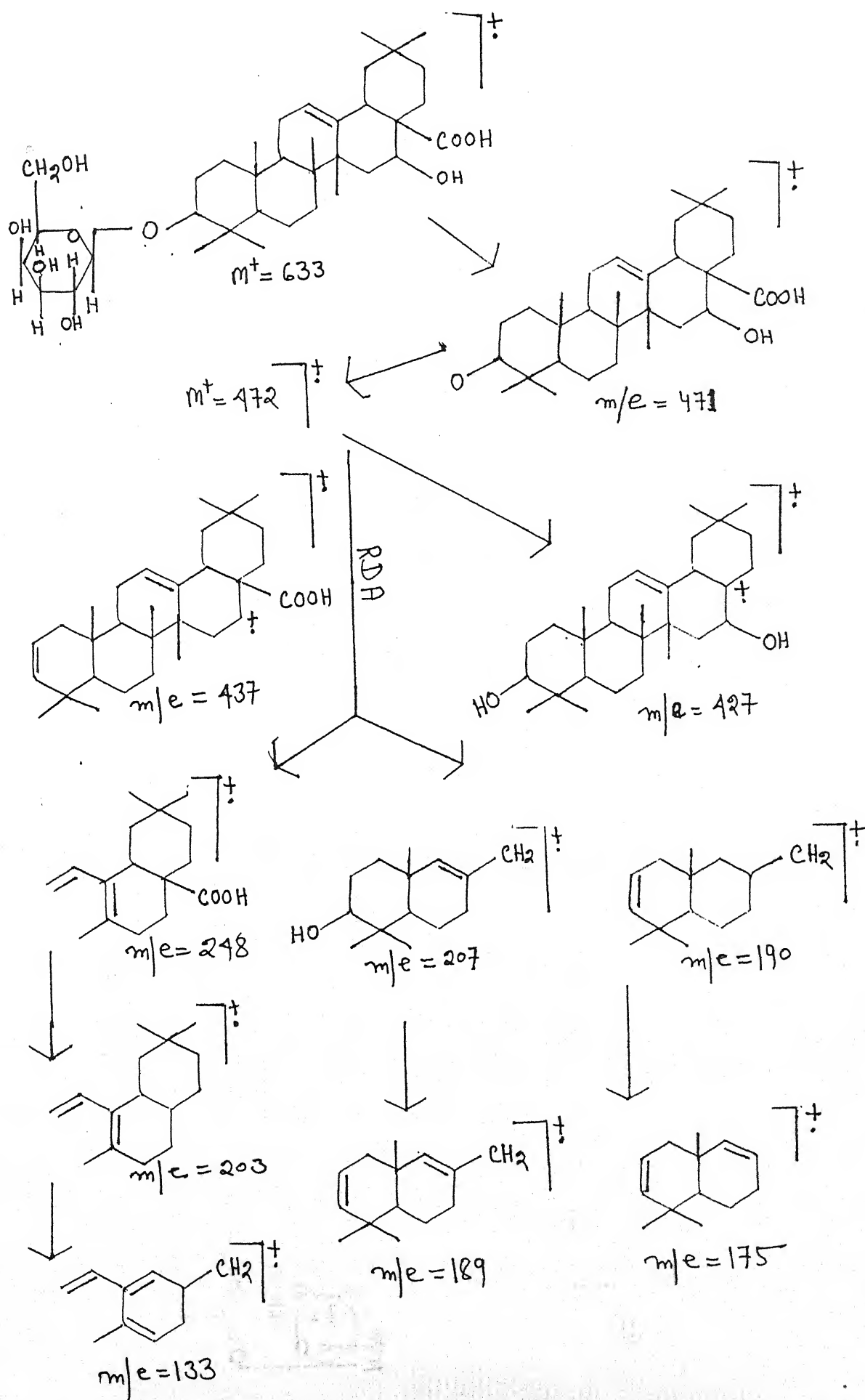
22.	34.1	C-22
23.	28.5	C-23
24.	17.2	C-24
25.	16.6	C-25
26.	17.8	C-26
27.	26.4	C-27
28.	176.8	C-28
29.	34.7	C-29
30.	23.7	C-30
31.	103.7	C-1'
32.	74.2	C-2'
33.	76.8	C-3'
34.	74.9	C-4'
35.	76.0	C-5'
36.	61.7	C-6'

#### MASS SPECTRUM OF THE COMPOUND TSG 1:

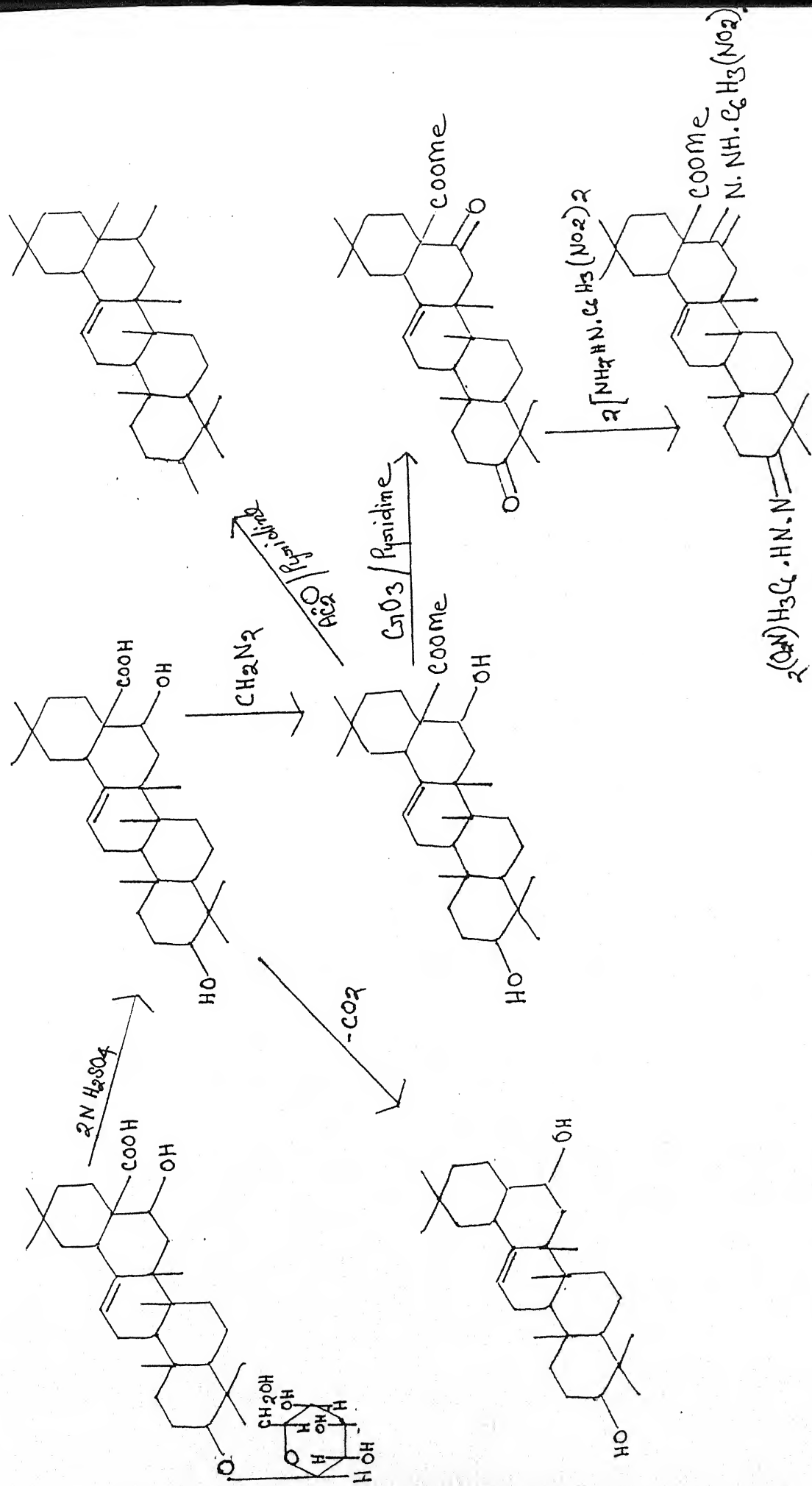
EIMS<sup>63-65</sup> of the saponin TSG 1 showed some significant fragmentation patterns, which are shown in the Scheme 2 and are summarised below:

M<sup>+</sup> 634 (EIMS) and the various fragmentation peaks at m/z 472, 471, 440, 427, 248, 207, 203, 190, 189, 175 and 133.

These spectral data further corroborated the structure of the compound as, Echinocystic acid -3-O- $\beta$ -D-galactopyranoside as shown below:



SCHEME-II



SCHEME-III

## EXPERIMENTAL

### EXTRACTION AND ISOLATION OF THE TRITERPENOIDAL SAPONIN:

The stems of *Ziziphus nummularia* were collected from the adjoining regions and were authenticated by the Botany Department of B.B. Science College Jhansi and a voucher specimen was deposited in this department. The plant material (stems of *Ziziphus nummularia*) were dried, finely powdered then extracted with 80% ethyl alcohol. The extract was concentrated under reduced pressure to get a dark brown viscous mass (850 mg.). This residue was partitioned between Et<sub>2</sub>O and water. The aq. layer was extracted with EtOAc followed by n- BuOH saturated with water. The n- BuOH fraction was subjected to chromatography on a silica gel column eluted with CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O in different ratios giving 46 fractions. Frs 21-36 were rechromatographed on a silica gel column eluted with CHCl<sub>3</sub>-EtOAc-Me OH (2:1:2) to afford compound TSG 1.

Column chromatography, results are shown below in Table 7:

TABLE 7

#### Column Descriptions:

I.	Length of the column	: 100 cm.
II.	Diameter of the column	: 3.5 cm.
III.	Amount of the Silica gel	: 100 g.
IV.	Weight of the ethyl acetate soluble part	: 5.72 g.

S. No.	Fractions	Solvents	Spots on TLC Plate	Substance
1.	1-10	CHCl <sub>3</sub> -MeOH- H <sub>2</sub> O (90:10:1)	mixture	Discarded
2.	11-20	80:20:2	Not remarkable	Discarded
3.	21-36	70:30:3	four	Further chromatograph ed
4.	37-46	60:40:4	mixture	Discarded

The fractions (21-36) were mixed together. The solvent was evaporated and residue obtained was further chromatographed on a silica gel column.

Colourless crystals of the compound TSG-1 were obtained on crystallisation with Me<sub>2</sub>CO. The saponin TSG-1 showed all the characteristic reactions of saponins.

#### STUDY OF TRITERPENOIDAL SAPONIN TSG 1 :

Compound TSG 1 was analysed for molecular formula, C<sub>36</sub>H<sub>58</sub>O<sub>9</sub> m.p. 251-53° and M<sup>+</sup> 634 (EIMS).

#### ELEMENTAL ANALYSIS OF TSG 1:

Found	Calculated value of elements
C = 68.36	C = 68.14 %
H = 9.01	H = 9.15 %

Molecular Weight: 634 (EIMS)

## **SPECIFIC TESTS OF SAPONINS:**

It responded positively to the following tests of saponins.

### **FOAM TEST:**

The compound formed a soapy layer of one-inch thickness, when it (10 mg) was mixed with 10 ml. of distilled water and shaken well. The soapy layer existed for a long period.

### **HAEMOLYTIC TEST:**

5.0 g. of gelatine was mixed with 100 ml. of saline solution (0.9 %) and the small amount of this solution was stirred with the 3.0 ml. of blood which was taken separately in a conical flask. Then this blood was applied on a glass plate and a drop of the extract was applied on this plate. The red blood gelatine became transparent after about one hour at the place of application of the extract. This test confirmed the presence of saponin in the extract.

### **METHYLATION OF SAPONIN TSG 1:**

The sapogenin TSG 1(40 mg.) was mixed with solvent ether and treated with an ethereal solution of diazomethane with simultaneous cooling when this reaction mixture was kept for approximately 10 hours, a yellow colour was appeared. After washing the mixture with  $H_2O$ ,  $NaHCO_3$  and drying it over anhydrous sodium sulphate, a residue was obtained. This residue was dissolved in the methanol, and was subjected to column chromatographed over silica gel, using  $MeOH:AcOH:Ac_2O$  (3:2:1) as eluting solvents when a homogenous methyl ester of saponin (30 mg.) was obtained.

**ELEMENTAL ANALYSIS OF METHYL ESTER OF COMPOUND TSG 1 (TSG 1e):**

**FOUND**

**CALCULATED**

C = 68.18 %

C = 68.52 %

H = 9.35 %

H = 9.26 %

Molecular Weight 648 (EIMS)

**ACETYLATION OF TSG 1e:**

The 25 mg of methyl ester of saponin was reacted with 10 ml of acetic anhydride and 10 ml of pyridine in a round bottomed flask fitted with reflux condenser on a water bath for 3 hours. The reaction mixture was cooled and poured into the ice cold water to get a precipitate. This ppt was extracted with ether in a separatory funnel. After washing the ethereal layer with sodium bicarbonate, the solvent was removed to yield a colourless acetylated derivative (18 mg) which was recrystallised with acetone.

**ELEMENTAL ANALYSIS OF THE ACETYLATED METHYL ESTER OF COMPOUND TSG 1 (TSG 1ac):**

**FOUND**

**CALCULATED**

C = 65.55 %

C = 65.73 %

H = 8.29 %

H = 8.16 %

% of acetyl group = 24.91%

% of acetyl group 25.06%

Molecular Weight 858 (EIMS)

## HYDROLYSIS OF THE COMPOUND TSG 1:

The saponin TSG 1 (900 mg) with 15 ml of ethanol, was taken in a round bottomed flask fitted with reflux condenser and 20 ml of 2 N sulphuric acid was mixed. The reaction mixture was refluxed for 5 hours on a water bath. The solution was concentrated under reduced pressure, cooled and the residue was extracted with solvent ether. After washing the ethereal layer with water, it was dried over anhydrous sulphate. Solvent ether was removed under reduced pressure to yield the sapogenin TSG 2 that was recrystallised with methanol to get colourless needles. The aqueous layer was neutralised and worked separately to identify the sugars present in the saponin TSG 1.

## STUDY OF SAPOGENIN TSG 2:

It was studied for molecular formula  $C_{30}H_{48}O_4$ , m.p.  $302^\circ$ , and  $M^+$  472 (EIMS). It responded all the reactions of triterpenoids and was soluble in Ethanol and feebly soluble in ethyl acetate.

## ELEMENTAL ANALYSIS OF SAPOGENIN TSG 2:

FOUND

C = 75.84

H = 10.09

Molecular Weight = 472 (EIMS).

CALCULATED

C = 76.23

H = 10.14

## COLOUR REACTIONS OF SAPOGENIN TSG 2:

### SALKOWASKI REACTION:

A yellow colour changing to red was produced when a conc.  $H_2SO_4$  was mixed to a solution of the sapogenin TSG 2 in chloroform.

### NOLLER'S REACTION:

A intense pink colour changing to violet was obtained when Noller's reagent (0.1%  $\text{SnCl}_2$  in thionyl chloride ) was reacted with a small amount of sapogenin TSG 2.

### TSCHUGAEV REACTION:

A violet red colour was produced when the solution of TSG 2 was mixed with acetyl chloride and boiled.

### PREPARATION OF METHYL DERIVATIVE (TSG 2c) OF SAPOGENIN:

Ethereal solution of diazomethane was mixed with constant cooling to the sapogenin (350 mg) solution in solvent ether, when this mixture was kept for about 10 hours, a yellow colour was produced. the mixture was washed with water and  $\text{NaHCO}_3$  solution and dried over anhydrous sodium sulphate. Residue obtained on removal of solvent was dissolved in methyl alcohol and subjected to column chromatography on Si - gel using  $\text{MeOH} : \text{AcOH} : \text{Me}_2\text{CO}$  3: 2: 1 as eluant, when crystals of methyl ester of sapogenin TSG 2c (310 mg.) were obtained by crystallisation with methyl alcohol. It was analysed for molecular formula  $\text{C}_{31}\text{H}_{50}\text{O}_4$ , m.p.  $221^\circ$  and  $\text{M}^+$  486 (EIMS).

### ELEMENTAL ANALYSIS OF METHYL ESTER DERIVATIVE OF SAPOGENIN (TSG 2c):

FOUND

C = 76.98

H = 10.37

CALCULATED

C = 76.54

H = 10.28

Molecular Weight = 486 (EIMS)

## FORMATION OF DIACETYL DERIVATIVE (TSG 2ac) OF METHYL ESTER DERIVATIVE OF SAPOGENIN (TSG 2e):

The 250 mg of methyl ester of sapogenin was reacted with 10 ml of acetic anhydride and 10 ml of pyridine in a round bottomed flask fitted with reflux condenser on a water bath for 4 hours. The reaction mixture was cooled and poured into the ice cold water to get a precipitate. This ppt was extracted with ether in a separatory funnel. After washing the ethereal layer with sodium bicarbonate, the solvent was removed to yield a colourless acetylated derivative (190-mg) which was recrystallised with acetone.

### ELEMENTAL ANALYSIS OF DIACETYL DERIVATIVE (TSG 2ac):

FOUND	CALCULATED
C = 74.10	C = 73.68
H = 9.57	H = 9.47

Molecular Weight = 570 (EIMS)

### OXIDATION OF THE METHYL ESTER OF SAPOGENIN (TSG 2e):

Methyl ester of sapogenin (TSG 2e) (25 mg) was dissolved in 5 ml of pyridine and to this solution, 15 mg. of  $\text{CrO}_3$  was added. This mixture was refluxed at  $120^\circ$  on a sand bath. This mixture was cooled and filtered, when a product (TSG 2op) was obtained which was dried to get a crystalline mass. It was analysed for mol. formula  $\text{C}_{31}\text{H}_{46}\text{O}_4$ , m.p.  $207^\circ$  and  $M^+$  482 (EIMS).

**ELEMENTAL ANALYSIS OF OXIDATION PRODUCT (TSG 2op) OF SAPOGENIN TSG 2:**

FOUND	CALCULATED
C = 76.67	C = 77.18
H = 9.37	H = 9.53

Molecular Weight = 482 (EIMS)

**ZIMMERMANN TEST:**

A violet colour was obtained when diketone derivative (TSG 2op) (5 mg) was dissolved in 2N KOH (10 ml) in absolute ethyl alcohol.

**FORMATION OF DIHYDROZONE DERIVATIVE OF TSG 2op:**

Diketone derivative (TSG 2op) (35 mg) was mixed with 25 ml of 2,4-dinitro phenyl hydrazine solution (prepared by dissolving 1.0 g. of solid 2,4-dinitro phenyl hydrazine in 250 ml of 2N HCl at 0-5° ) in a glass stoppered dry flask with vigorous shaking and then cooling it on an ice bath for 2 hours till a ppt was obtained. After washing it with 2N HCl and H<sub>2</sub>O, was crystallised from EtOH to yield di (2,4-dinitro phenyl hydrazine) derivative.

**ELEMENTAL ANALYSIS OF DIHYDROZONE DERIVATIVE OF (TSG 2op):**

FOUND	CALCULATED
C = 60.88	C = 61.27
H = 6.51	H = 6.42
N = 13.09	N = 13.29

Molecular Weight = 842 (EIMS)

### SAPONIFICATION OF METHYL ESTER (TSG 2e):

The monomethyl ester (TSG 2e) (25 mg) of sapogenin (TSG 2) was taken in a round bottomed flask with ethylene glycol (20 ml) and 2N KOH (30 ml). The reaction mixture was refluxed on sand bath at 150° for four hours. After cooling, it was transferred to ice cold water with constant stirring and then extracted with solvent ether to obtain a gelatinous mass. It was recrystallised from methyl alcohol to get colourless needles.

### STUDY OF SUGAR MOIETY:

After acid hydrolysis of saponin TSG 1, the hydrolysate obtained was neutralised by the addition of barium carbonate and barium sulphate. This hydrolysate was then filtered off and the filtrate was then concentrated under reduced pressure and paper chromatography was applied to this concentrate by using various solvent systems, which are given in Table 8 and aniline hydrogen phthalate as spraying reagent.

Table 8

S. No.	Solvent System	Rf. Value (recorded)	Rf. Value ( found )	Sugar Identified
1.	n BuOH : AcOH : Water ( 4:1:5 )	0.16	0.17	D-galactose
2.	EtOAc : Water : Pyr. ( 2 : 2 : 1 )	0.235	0.24	D- galactose

### PERMETHYLATION AND HYDROLYSIS OF SAPONIN TSG 1:

The saponin TSG 1 was subjected to the permethylation by treating it (20 mg.) with MeI (1.0 ml.), silver oxide (16 mg.) in dimethyl formamide (2.0 ml.) for 48 hours at room temperature, then the mixture was filtered off and the residue was taken in  $\text{CHCl}_3$  (12 ml.) and the  $\text{CHCl}_3$  layer was taken with dimethyl formamide. The solvent was removed to get a syrupy mass. This syrupy mass was then hydrolysed by using Killiani's mixture (HCl: AcOH: Water 5: 12: 18). On addition of excess of water to the mixture, the methylated sapogenin was precipitated out. The aq. part was neutralised with barium carbonate and barium sulphate formed was filtered off. On TLC of concentrated filtrate with toluene: methyl alcohol (4:1) as solvent system, the methylated sugar was identified as 2,3,4,6 tetra - O -methyl - D- galactose. (Confirmed by Co-PC and Co-TLC).

### ENZYMATIC HYDROLYSIS OF THE COMPOUND TSG 1:

The saponin TSG 1 (25 mg.) was taken in alcoholic medium and reacted with almond emulsin solution and mixture was left over night at room temperature. The ppt obtained was filtered off and the hydrolysate was subjected to paper chromatography using n- BuOH: AcOH: Water (4: 1: 5) as solvent system and aniline hydrogen phthalate as spraying reagent when a single spot appeared.

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## CHAPTER V

.....  
ISOLATION AND IDENTIFICATION OF A TRITERPENOIC, "3, 21-  
DIHYDROXY - OLEAN - 12 - EN - 28 - OIC ACID" FROM THE AERIAL  
PARTS OF *CELSIA COROMANDELIANA*"  
.....

*Celsia coromandeliana*<sup>1</sup> Vahl. (Family Scrophulariaceae) is a weed in cultivated grounds that occurs throughout India from Punjab to Ceylon and ascending to 5000 ft. on the Himalayas extending to Afghanistan, Burma and China.

An erect pubescent, annual herb, stems 2-3 ft. high, glandular above. Radical leaves, petiolated, crowded, 2-4 inch long, the large terminal lobe usually oblong lyrate-pinnatifid; the large terminal lobe usually oblong, obtuse, toothed: sessile, smaller and passing upwards into bracts, oblong-ovate, cordate, toothed, hairy on both sides. Flowers in simple and branched terminal racemes, pedicels 1/4 to 1/3 inch long; bracts shorter, ovate. Calyx shorter than the pedicels deeply divided; segments linear-oblong, subacute, entire or serrulate. Corolla 1/2 in. across, yellow. Filaments densely bearded with purple hairs. Capsule 1/4-1/3 inch in diameter, subglobose, glabrous. Seeds oblong, truncate, verrucose.

In Sanskrit, the plant is named as *Bhutakeshi & Kulahala* and in Hindi, it is called as *Gadartambaku*. The juice of the leaves of this plant is sedative, astringent and is used in diarrhoea and dysentery. The plant juice is used in the skin eruptions and fevers<sup>2</sup>.

*Celsia coromandeliana* is only available species of genus *Celsia* in India. It has been reported to contain celsianol<sup>3</sup>, a sterol and three triterpenoid saponins, named as celsiosides<sup>4</sup>, I, II and III which later furnished celsiogenin C (a new genin) with celsiogenin A & B<sup>5</sup>.

Therefore, in hope of the exploration of some compounds, which can define its medicinal properties, the author decided to undertake its detailed and systematic phytochemical study.

## SEPARATION OF TRITERPENOIDAL COMPOUND (TC):

The air-dried and powdered aerial parts of *Celsia coromandeliana* were extracted with methyl alcohol under reflux. The extract was diluted with water and then extracted at room temperature with chloroform, ethyl acetate and n- butyl alcohol successively.

## STUDY OF CHLOROFORM SOLUBLE FRACTION:

The  $\text{CHCl}_3$  extract was defatted with n- hexane. The portion insoluble in hexane was chromatographed on a silica gel column. The fraction obtained on eluting the column with  $\text{CHCl}_3$ :  $\text{CH}_3\text{OH}$  (97:3), showed a single spot on TLC examination and responded positively to the tests of triterpenoids<sup>6,7</sup>. On removal of solvent, a white-cream amorphous mass was obtained, which on TLC examination was found homogeneous and on crystallisation with methanol, yielded colourless; needle shaped crystals of the compound TC, which responded positively to the tests of triterpenoids.

## STUDY OF THE TRITERPENOID TC:

The triterpenoid TC was analysed for the molecular formula  $\text{C}_{30}\text{H}_{48}\text{O}_4$ , m.p.292-293° and  $\text{M}^+$  472 (EIMS). It was soluble in methanol and ethanol and gave all the tests of triterpenoids.

## PENTACYCLIC NATURE OF TRITERPENOID:

The triterpenoid compound TC was pentacyclic in nature, because it exhibited violet colour with 2, 6-di tertiary-butyl-p-cresol in ethanol<sup>8</sup>.

## UV SPECTRUM OF THE COMPOUND TC:

In the UV spectrum of compound TC, no absorption beyond 188 nm was observed.

## IR SPECTRUM OF THE COMPOUND TC:

The IR spectra<sup>9-11</sup> of compound TC showed some characteristic peaks that were utilised in inferring some important structural units which are given in the Table 1 (Fig 1).

Table 1

Peaks	Structural Assignments
3575	-OH group
3032, 1628	Double bond
2960	CH stretching vibration of Me group
2895	-CH stretching in pentacyclic nucleus
2858	Presence of Me group
1720	-COOH group
1634	-CH <sub>2</sub> -CH
1385, 1374	Gem dimethyl group
1396, 1380, 1320	Triterpenoidal nucleus
1370	-CH bending of methyl group
823	CH = CH <sub>2</sub>

Therefore, on basis of these facts, following tentative structure of compound TC can be given as follows:

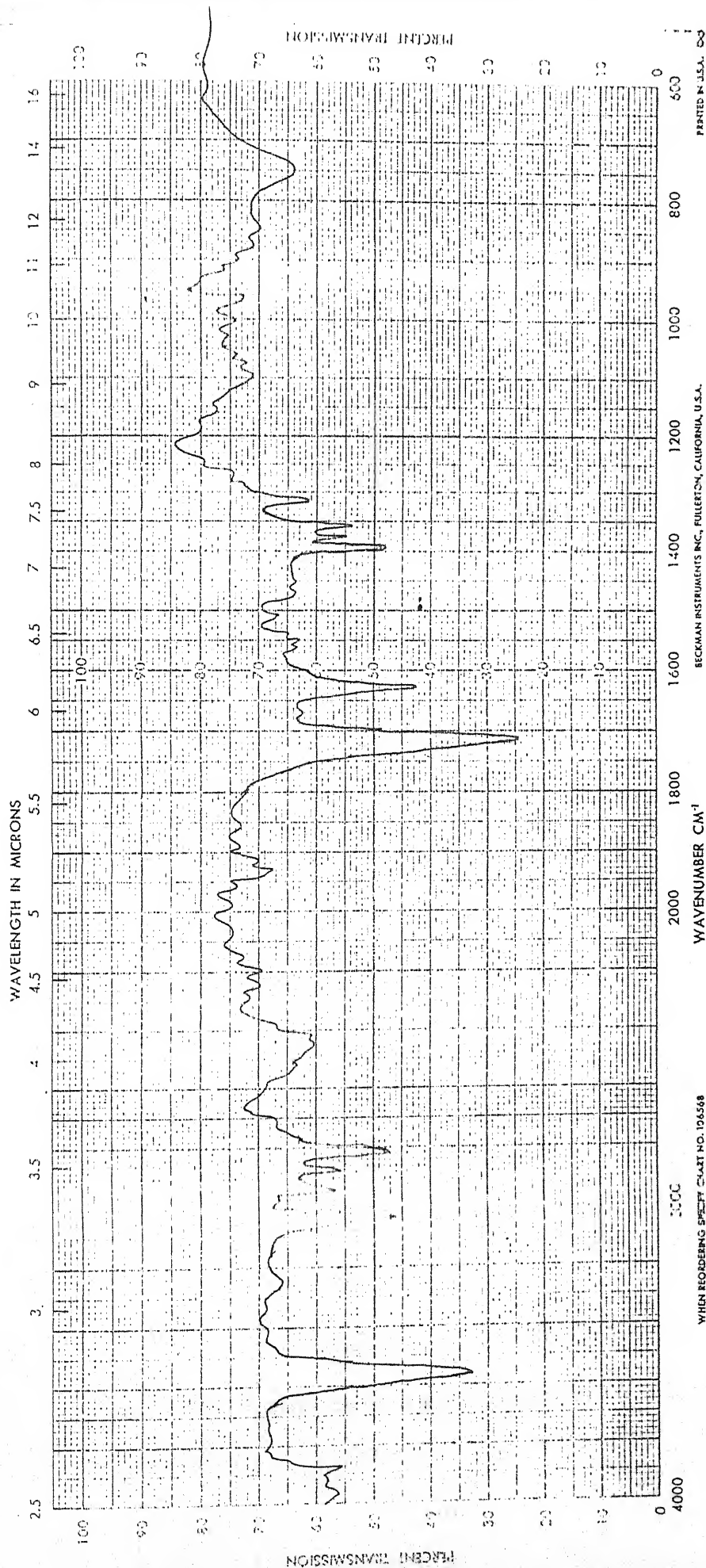
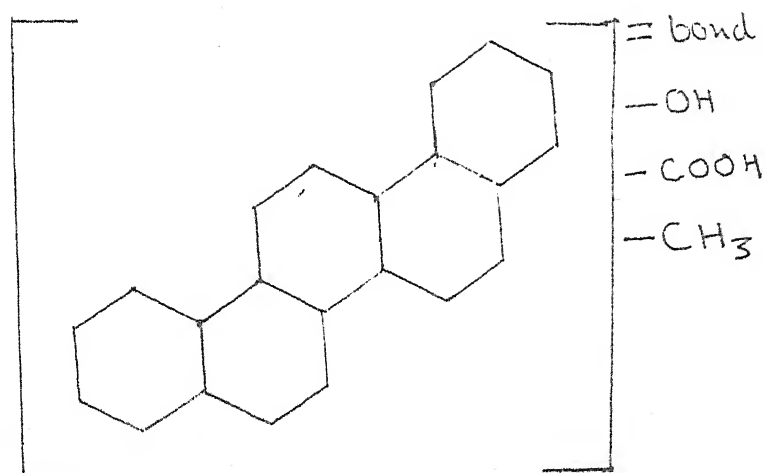


Fig. 1



### PRESENCE OF DOUBLE BOND:

In the IR spectrum of the compound TC peaks at  $\nu_{\text{max}}^{\text{KBr}}$  3032, 1628  $\text{cm}^{-1}$  pointed towards the presence of double bond. The presence of a double bond was further corroborated by the yellow coloration with TNM.

### POSITION OF DOUBLE BOND:

The unreactivity of the compound with  $\text{H}_2/\text{Pt}$  at  $280^\circ$  suggested the presence of resistant double bond inside the ring system. The presence of double bond at 12-13 position was suggested by the high terminal absorption at 185 nm in the UV spectrum, which is a characteristic peak of double bond at this position in oleanane series of majority of triterpenes<sup>12</sup>. This was further confirmed by the upfield NMR peak at  $\delta$  5.24 for the vinylic proton at C -12, a downfield peak at  $\delta$  2.15 for the  $\text{CH}_2$ -11 and another downfield peak at  $\delta$  2.40 for the C-18 proton<sup>13</sup>.

### PRESENCE OF -COOH GROUP:

A peak at  $\nu_{\text{max}}^{\text{KBr}}$  1720  $\text{cm}^{-1}$  in the IR spectrum indicated the presence of -COOH group. Effervescence on treatment of compound TC with sodium bicarbonate

solution further proved this fact. The formation of a methyl ester TCme (analysed for molecular formula  $C_{31}H_{50}O_4$ , m.p.  $193^\circ$  and  $M^+$  486(EIMS) on methylation with  $CH_2N_2$ , confirmed the presence of carboxylic group.

#### POSITION OF $-COOH$ GROUP:

On heating triterpenoid, TC gave a decarboxylated product, TCdy (analysed for molecular formula  $C_{29}H_{48}O_2$ , m.p.  $180^\circ$ ,  $M^+$  428, (EIMS). In the  $^{11}H$  NMR spectrum, decarboxylated triterpenoid TCdy, showed a signal at  $\delta$  2.54 (m, 1H) for H-17 proton, while, no such peak for H-17 was observed in the spectra of mono methylated diacetyl derivative (TCac), thus, proving the presence of  $-COOH$  group at C-17 position, which was further confirmed by the  $^1H$  NMR peak at  $\delta$  3.66 (s, 3H) for  $-COOMe$  group<sup>12,14</sup> of its mono methylated diacetylated derivative.

#### PRESENCE OF $-OH$ GROUP (S):

In the Infra Red spectrum of the triterpenoid TC, emergence of a peak at  $\nu_{max}^{KBr}$   $3575\text{ cm}^{-1}$  showed the presence of  $-OH$  groups. No. of OH groups were determined by acetylation of its monomethyl ester (TCme) with  $Ac_2O/pyr$ , when it yielded an acetylated product (TCac) with molecular formula  $C_{35}H_{54}O_6$ , m.p.  $258^\circ$ ,  $M^+$  570 EIMS. By using the Weisenberger process as described by Belcher and Godbert<sup>15</sup>, compound TCac showed two acetyl groups (14.85%), thus, confirming the presence of two  $-OH$  groups in the compound TC.

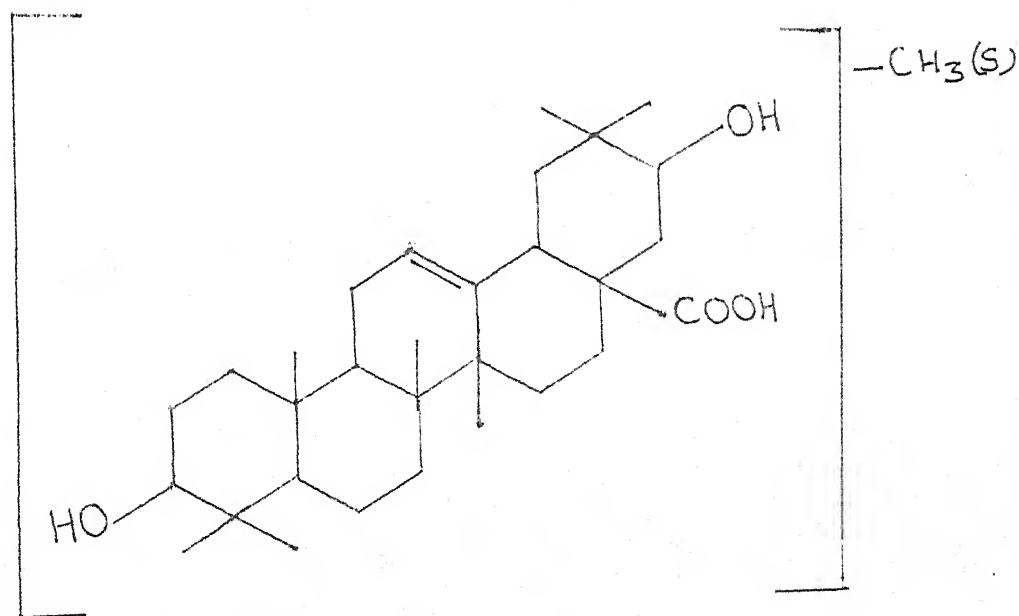
#### POSITION AND NATURE OF $-OH$ GROUPS:

In the  $^1H$  NMR spectrum of the diacetylated monomethyl ester derivative (TCac), a peak at  $\delta$  3.22 (1H, dd,  $J = 10.2, 9.0\text{ Hz}$ , Carbinyllic proton at H-21), another peak at  $\delta$  4.03 (1 H, dd,  $J=10$  and  $5\text{ Hz}$ , Methenic proton at H-3) and two singlets

at  $\delta 2.01$  (3H, C-3, -OAc) and  $\delta 2.10$  (3H, C-21 OAc) indicated the position of -OH groups at C-3 and C-21 and their secondary nature.

Monomethyl ester of compound TC gave an oxidation product (TCop); molecular formula  $C_{31}H_{48}O_4$ , m.p.  $162-163^\circ$ ,  $M^+$  484 (HIMS), when it was oxidized with  $CrO_3$  / pyridine. Compound TCop gave dihydrazone derivative on reaction with 2,4-dinitro phenyl hydrazine, thus, confirming the oxidation product to be diketo derivative and indicated towards the secondary nature of both the -OH groups of the triterpenoid TC. The Zimmermann test<sup>16</sup> (a specific test for C-3 keto group) of the compound TCop gave positive results, which further corroborated the position of one keto group at C-3 position. Therefore, OH groups were assigned at C-3 and C-21 in the compound TC.

A tentative structure to the compound TC is given on the basis of positions of -OH groups as below :



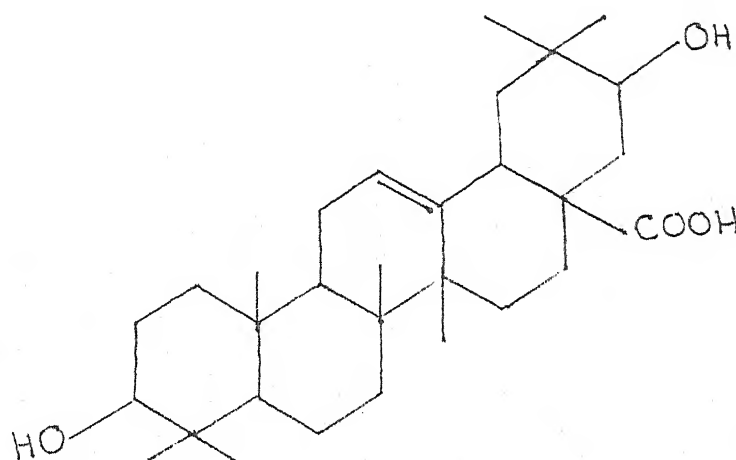
### PRESENCE OF METHYL GROUP(s) :

In the IR spectrum of compound TC, peaks at  $\nu_{\text{max}}^{\text{KBr}}$  2858 and 1370  $\text{cm}^{-1}$  indicated the presence of methyl group(s).

### POSITION OF METHYL GROUPS:

The  $^1\text{H}$  NMR peaks at  $\delta$  0.77, 0.86, 0.93, 0.96, 0.98, 1.02 and 1.08 (each 3H, s) of acetylated derivative (TCac) were in accordance with the oleanane type ( $\beta$  amyrin) of skeleton<sup>17-18</sup>. Thus, the positions of seven methyl groups were established at C<sub>23</sub>, C<sub>24</sub>, C<sub>25</sub>, C<sub>26</sub>, C<sub>27</sub>, C<sub>29</sub> and C<sub>30</sub>.

Taking the above facts in the consideration the structure of triterpenoid TC was given as (I): 3, 21-dihydroxy - olean - 12 - en - 28 - oic acid which is given blow:



The above structure of compound TC was found to be in complete conformity with its IR,  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR and Mass spectral studies.

**$^1\text{H}$  NMR SPECTRUM OF DIACETYLATED METHYL ESTER OF THE TRITERPENOID (TCac):**

In the  $^1\text{H}$  NMR spectrum of diacetylated methyl ester of TC, some important peaks were obtained, which were interpreted to establish the structural assignments with the help of available literature<sup>19,20</sup>, which are given in the Table 3 (Fig 2).

**Table 2**

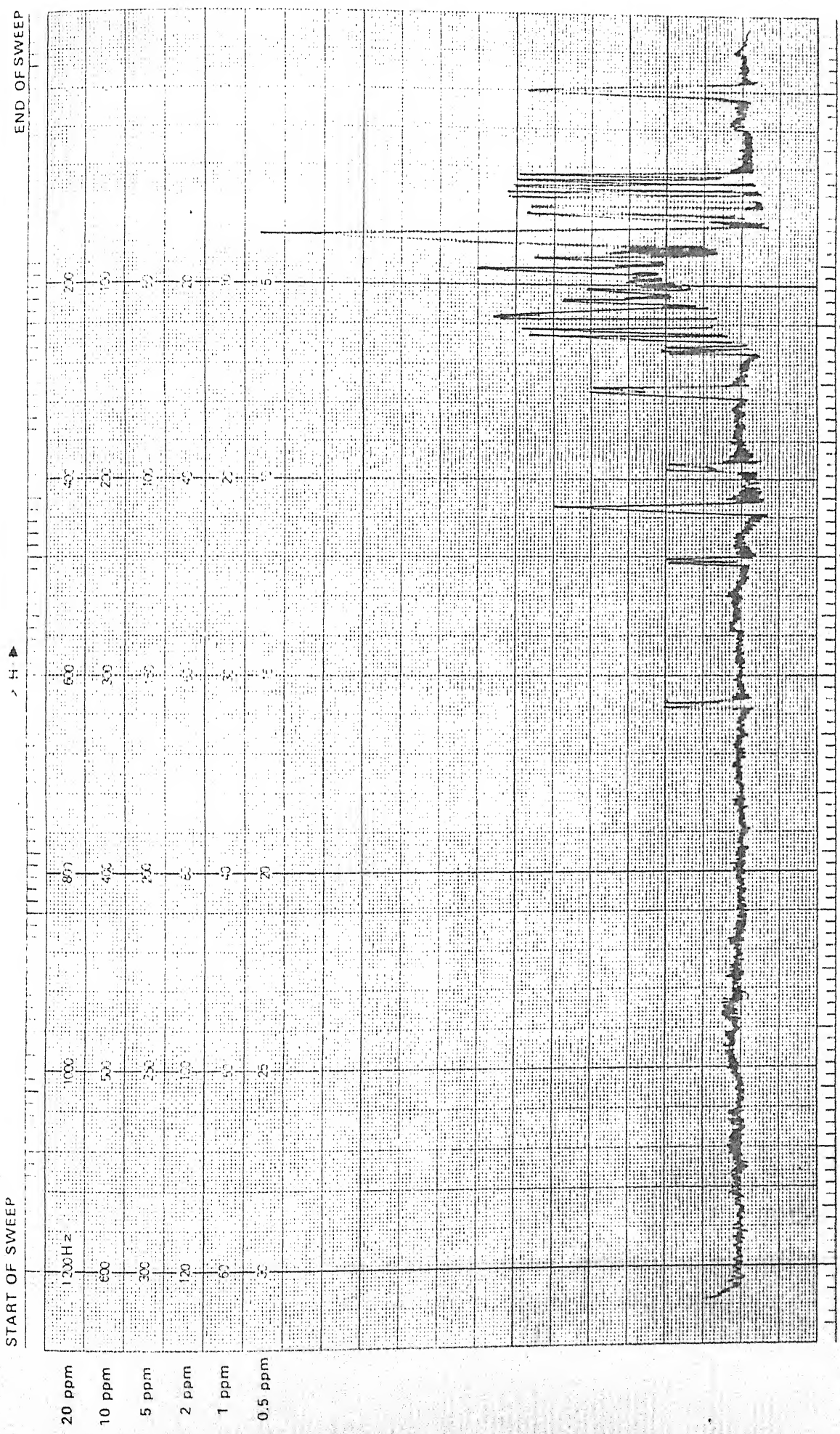
S. No.	$\delta$ Value	No. of protons	Pattern	J Value in Hz	Structural Assignments
1.	0.77	3	s		Me group
2.	0.86	3	s		Me group
3.	0.93	3	s		Me group
4.	0.96	3	s		Me group
5.	0.98	3	s		Me group
6.	1.02	3	s		Me group
7.	1.08	3	s		Me group
8.	1.22-1.98	18	m	-	Polymethyl- ene and methyl $\text{CH}_2$ and CH
9.	2.01	3	s	-	C-3 OAc

10.	2.10	3	s	-	C-21 OAc
11.	2.15	2	t	-	CH <sub>2</sub> -11
12.	2.51	1	dd	3.6, 9.6	H-18
13.	3.22	1	dd	9.0, 10.2	Carbinyllic proton at C- 21
14.	3.66	3	s	-	COOMe at C-17
15.	4.03	1	dd	5, 10	Methine proton at C-3
16.	5.24	1	dd	6.9, 10.1	Vinyllic proton at C-12

### **<sup>13</sup> C NMR SPECTRUM OF THE COMPOUND TC:**

In the <sup>13</sup> C NMR spectrum of triterpenoid TC, significant signals were observed, which were interpreted with the help of available literature<sup>21</sup> The signals obtained and the structural assignments inferred with the help of this spectra are given in Table 3:

# EM-360 60 MHz NMR SPECTROMETER



LOCK POS. \_\_\_\_\_ ppm

LOCK POWER \_\_\_\_\_ mG

DECOUPLE POS. \_\_\_\_\_ ppm

DECOUPLE POWER \_\_\_\_\_ mG

FILTER \_\_\_\_\_

RF POWER \_\_\_\_\_ mG

SPECTRUM AMPL. \_\_\_\_\_

Sweep TIME \_\_\_\_\_ min

Sweep WIDTH \_\_\_\_\_ ppm

END OF SWEEP \_\_\_\_\_ ppm

NUCLEUS \_\_\_\_\_

SAMPLE \_\_\_\_\_

OPERATOR \_\_\_\_\_

DATE \_\_\_\_\_

SAMPLE TEMP. \_\_\_\_\_ °C

SOLVENT \_\_\_\_\_

SPECTRUM NO \_\_\_\_\_

Fig. 2

Table 3

S. No.	$\delta$ Value	Assignment
1.	39.9	C-1
2.	28.7	C-2
3.	77.4	C-3
4.	39.8	C-4
5.	56.6	C-5
6.	17.9	C-6
7.	31.7	C-7
8.	38.8	C-8
9.	46.5	C-9
10.	37.6	C-10
11.	24.3	C-11
12.	123.2	C-12
13.	145.1	C-13
14.	42.9	C-14
15.	27.6	C-15
16.	22.6	C-16
17.	47.5	C-17
18.	41.7	C-18
19.	47.3	C-19
20.	31.1	C-20
21.	62.4	C-21

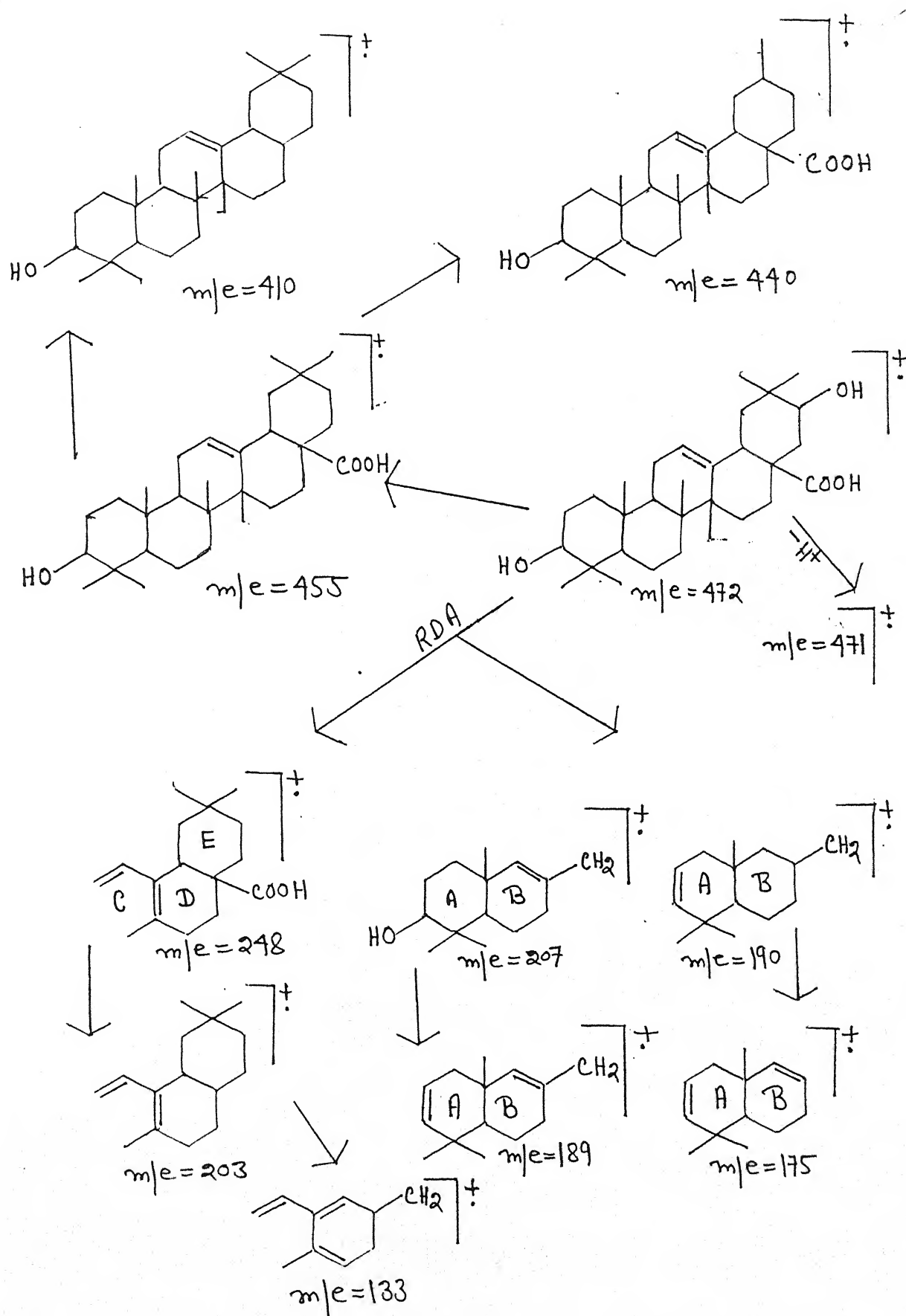
22.	34.4	C-22
23.	27.5	C-23
24.	17.1	C-24
25.	15.1	C-25
26.	16.1	C-26
27.	25.0	C-27
28.	178.4	C-28
29.	34.9	C-29
30.	22.6	C-30

#### MASS SPECTRUM OF THE COMPOUND TC :

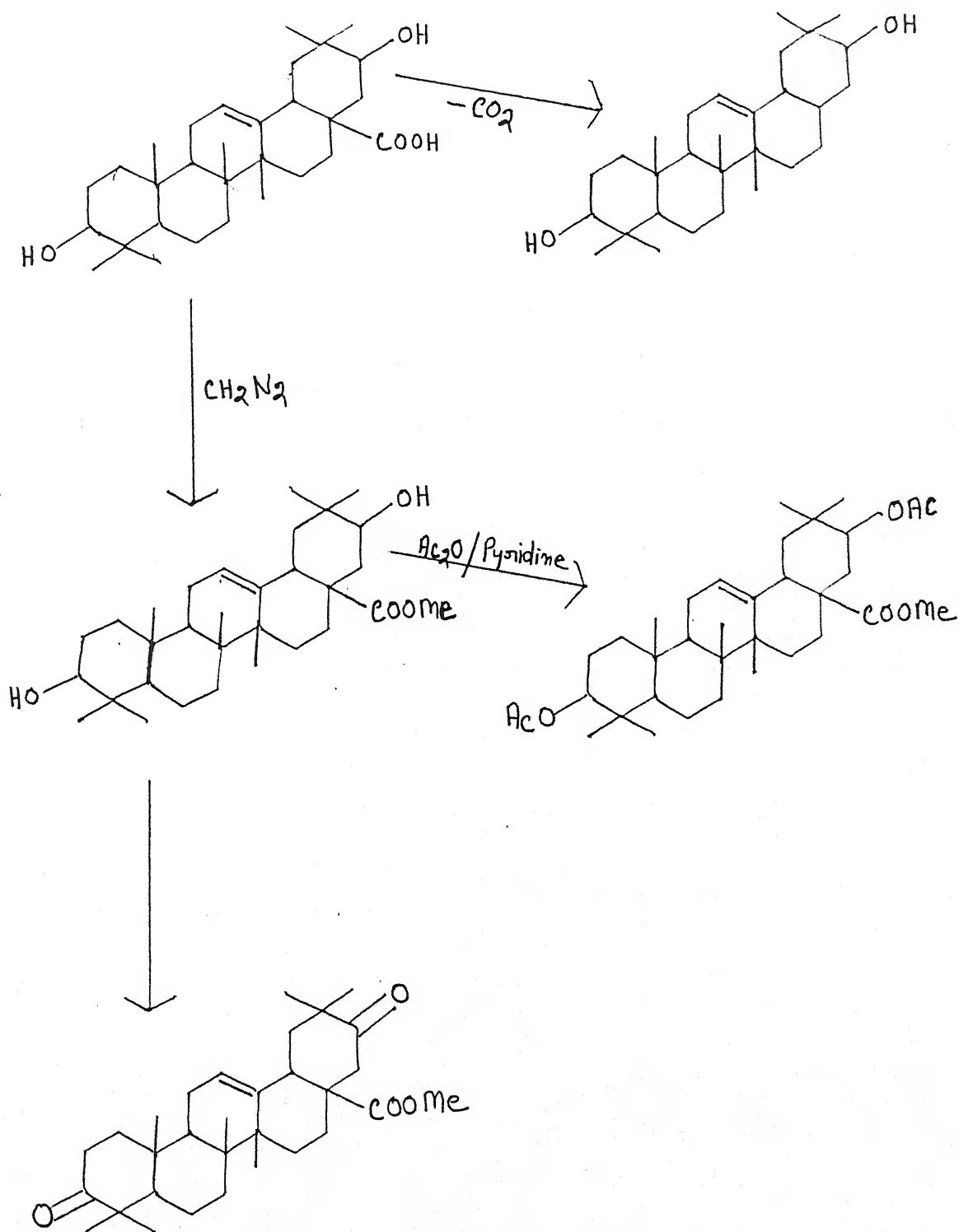
The mass spectra<sup>22-24</sup> of the compound TC further supported the structure I of the compound TC. Important fragmentation patterns obtained in the electron impact mass spectrum (EIMS) are given as below:

$M^+ = 472$   $M/z = 471, 455, 440, 410, 248, 207, 203, 190, 189, 175$  and  $133$ .

The different fragmentation patterns obtained in the mass spectra of the compound TC are shown in the scheme I.



SCHEME-I



SCHEME-II

## EXPERIMENTAL

### EXTRACTION AND ISOLATION OF THE TRITERPENOIDAL COMPOUND:

The aerial parts of *Celsia coromandeliana* were collected from the adjoining regions and were authenticated by the Botany Department of B.B. Science College Jhansi and a voucher specimen (No. HMG 1002) was deposited in the same department. 4.0 kg of finely powdered, plant material (aerial parts of *Celsia coromandeliana*) was extracted with methyl alcohol in a flask fitted with reflux condenser. The extracts were diluted with  $H_2O$  and then extracted at room temperature with  $CHCl_3$ , EtOAc and n- BuOH successively. The  $CHCl_3$  extract was defatted with hexane. The hexane insoluble portion was chromatographed on a silica gel column. This column was then run successively with  $CHCl_3$ :  $CH_3OH$  in different proportions. The  $CHCl_3$ :  $CH_3OH$  (97:3) portion showed a single spot on TLC examination using  $CHCl_3$ :  $CH_3OH$  (9:1) solvent system and 10% sulphuric acid as visualising agent. The details of column chromatography are given in the Table 4.

Table 4

#### Column Descriptions:

I.	Length of the column:	120 cm.
II.	Diameter of the column:	3.0 cm.
III.	Amount of the Silica gel	: 110 g.
IV.	Weight of the mass	: 2.72 g.

S. No.	Fractions	Solvents	Spots on TLC	Substance
		Chloroform:	Plate	
		Methanol:		
1.	1-8	99:1	4	Mixture of 4 compounds and rejected.
2.	9-16	97:3	1	White cream amorphous compound.
3.	17-24	95:5	3	Mixture of three comps.  Rejected

The Rf. value of fractions (9-16) was found to be same, thus, they were mixed together. After evaporating the solvent, a white-cream amorphous residue was obtained which was examined by TLC. It was purified by crystallising it with methanol when it yielded colourless; needle shaped crystals of the compound TC. The compound TC showed the entire characteristic reactions of Triterpenoids.

#### STUDY OF TRITERPENOID TC:

It was studied for molecular formula  $C_{30}H_{48}O_4$ , m.p. 292-293°, and  $M^+$  472 (EIMS). It gave all the characteristic reactions of triterpenoids and was soluble in methanol.

## **ELEMENTAL ANALYSIS OF TRITERPENOID TC:**

### **FOUND**

C = 76.41

H = 10.28

Molecular Weight = 472 (EIMS).

### **CALCULATED**

C = 76.26

H = 10.17

## **COLOUR REACTIONS OF TRITERPENOID TC:**

It gave all the characteristic colour reactions.

### **SALKOWASKI REACTION:**

This test was performed in the similar manner as described in Chapter 4 on page no. 134 of this thesis.

### **NOLLER'S REACTION :**

The procedure adopted for this test was similar as described in the Chapter 4 on page no. 135 of this thesis.

### **TSCHUGAEV REACTION :**

This test was done in the similar way as reported in Chapter 4 on page no. 135 of this thesis.

## **PREPARATION OF METHYL DERIVATIVE (TC<sub>me</sub>) OF TRITERPENOID :**

To the 300 mg of triterpenoid TC solution in ether, ethereal solution of diazomethane was added with constant cooling until a yellow colour was produced. The reaction mixture was washed with water and sodium carbonate solution and dried over anhydrous sodium sulphate. The solvent was removed and the residue obtained was subjected to column chromatography on Si-gel-using MeOH: AcOH (3:1) as eluant. The homogenous residue obtained was recrystallised with methanol to get 260 mg of methyl

ester derivative (TCme) of triterpenoid TC. The molecular formula and m.p. 193° of TCme were found to be  $C_{31}H_{50}O_4$  respectively.

#### ELEMENTAL ANALYSIS OF METHYL ESTER (TCme) :

FOUND	CALCULATED
C = 76.86	C = 76.54
H = 10.40	H = 10.29

Molecular Weight = 486 (EIMS)

#### PREPARATION OF DIACETYL DERIVATIVE (TCac) OF METHYL ESTER DERIVATIVE OF TRITERPENOID (TCme):

The acetylation of methyl ester of triterpenoid was performed by taking 200 mg of TCme with 10 ml of acetic anhydride and 10 ml of pyridine in a round bottomed flask fitted with reflux condenser and refluxing the contents on a water bath for about 4 hours. Then reaction mixture was cooled and poured into the ice cold water to get a precipitate, which was extracted with ether in a separatory funnel. The ethereal layer was washed with sodium bicarbonate, dried and the solvent was removed to yield a colourless acetylated derivative (165 mg ) which was recrystallised with acetone. It was analysed for the molecular formula  $C_{35}H_{54}O_6$ , m.p. 258° and  $M^+$  570(EIMS)

#### ELEMENTAL ANALYSIS OF TCac:

FOUND	CALCULATED
C = 73.97	C = 73.68
H = 9.55	H = 9.48

Molecular Weight = 570 (EIMS)

### OXIDATION OF THE METHYL ESTER OF TRITERPENOID (TCme):

The methyl ester of triterpenoid, TCme (30 mg) was oxidised by taking it with 10 ml of 80% acetic acid in a round bottomed flask and a solution of 200 mg of  $\text{CrO}_3$  in 10 ml of pyridine was added to this reaction mixture with constant cooling till a fast orange colour was obtained. The oxidation product (TCop) was obtained after filtration that was dried to get a crystalline mass. It was analysed for mol. formula  $\text{C}_{31}\text{H}_{46}\text{O}_4$ , m.p.  $162-163^\circ$  and  $M^+$  482 (EIMS) and responded positively to the Zimmermann test for keto group presence.

### ELEMENTAL ANALYSIS OF OXIDATION PRODUCT OF TRITERPENOID TC (TCop):

#### FOUND

C = 76.91

H = 9.55

#### CALCULATED

C = 77.17

H = 9.54

Molecular Weight = 482 (EIMS)

### ZIMMERMANN TEST:

This test was performed in a similar manner as reported in Chapter 4 on page no. 137 of this thesis.

### FORMATION OF DIHYDRAZONE DERIVATIVE OF TCop:

Dihydrozone derivative of TCop was formed in a similar manner as described in Chapter 4 on page no. 137 of this thesis

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## APPENDIX

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COPIES OF ACCEPTED AND COMMUNICATED RESEARCH PAPERS

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# PHYTOCHEMISTRY

*Chemistry    Biochemistry    Molecular Biology*

Editor: UK, Africa & the Commonwealth  
Prof. G. P. Bolwell

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*Phytochemistry*  
329b Bourne Building  
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Wednesday, Sep 20, 2000

Dr. Harimohan M Gupta  
Department of Chemistry  
Bipin Bihari Science College  
Jhansi. 284 001 (UP)  
India

**RE: Manuscript #UK010/00**

Dear Dr. Gupta.

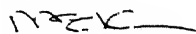
Thank you for submitting your manuscript entitled

"6-[2''-Hydroxy-3''-methyl butyl] quercetin-7-O-(2'''-galloyl)-beta-D-glucopyranoside  
from *Kickxia ramosissima*"

to *Phytochemistry*. I have assumed responsibility for its editorial review. Please include a  
reference to the manuscript number #UK010/00 in all communications with the journal  
offices regarding your paper.

Our recorded date of submission is 09/19/2K. You will be hearing from us as soon as  
possible with regards to the outcome of the review process.

Sincerely yours.



Prof. G. Paul Bolwell

Editor

6 - [2'' - HYDROXY - 3'' - METHYL BUTYL] QUERCETIN - 7 - O - (2''' - GALLOYL) -  
β - D - GLUCOPYRANOSIDE FROM *KICKXIA RAMOSISSIMA*

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**Key Word Index :-** *Kickxia ramosissima*, *Linaria ramosissima*; Scrophulariaceae ; 6 - [2'' - hydroxy - 3'' - methyl butyl] quercetin - 7 - O - (2''' - galloyl) - β - D - glucopyranoside, quercetin glycoside.

**Abstract :-**

The ethyl acetate soluble fractions of aerial parts of *Kickxia ramosissima* (Wall.) Janchen Syn. *Linaria ramosissima* Wall. (Scrophulariaceae) yielded a micro-crystalline compound identified as 6 - [2'' - hydroxy - 3'' - methyl butyl] quercetin - 7 - O - (2''' - galloyl) - β - D - glucopyranoside by spectral analysis and chemical reactions.

**INTRODUCTION**

*Kickxia ramosissima* (Wall.) Janchen syn. *Linaria ramosissima* (fam. Scrophulariaceae) occurs throughout India in cracks and fissures of old buildings and is used in the treatment of hyperglycemia. [1]

In current phytochemical investigations on local plants, the authors, herein, report the presence of a novel flavonoid glycoside, 6 - [2'' - hydroxy - 3'' - methyl butyl] quercetin - 7 - O - (2''' - galloyl) - β - D - glucopyranoside, identified on the basis of spectral and chemical evidences.

## RESULTS AND DISCUSSION

Compound 1 ( $C_{33}H_{34}O_{17}$ ) obtained as a yellowish microcrystalline substance, showing +ve Shinoda test [2] and Molisch's test for flavonoids and sugars respectively indicating flavonoidal and glycosidic nature of compound. The UV  $\lambda_{\text{max}}^{\text{MeOH}}$  267 and 368 nm indicated a flavonoidal skeleton. The IR peak  $3400\text{ cm}^{-1}$  pointed towards the presence of OH group(s). The peracetylation of compound 1 ( $\text{NaOAc} + \text{Ac}_2\text{O}$ ) yielded a derivative which was found to be undecaacetate by the Wisenberger method, due to 11 OH groups. The appearance of chemical shifts at  $\delta$  2.01 – 2.59 integrating for 33 protons, which confirmed the presence of 11 OH groups in the molecule.

### Presence of Acylation

The downfield chemical shift in  $^1\text{H}$  NMR spectrum of glycoside 1 at  $\delta$  5.10 was an indication of acylation [3]. It was confirmed by alkaline hydrolysis (2%NaOMe) [4], yielding methyl ester of gallic acid and the compound 2. Permethylation of 1 followed by acid hydrolysis led to the conclusion that the attachment of gallic acid was at C-2''' of D-glucose. In the IR spectrum of glycoside 1, a peak at  $1715\text{ cm}^{-1}$  exhibited the C=O stretching absorption band of ester group, pointing towards the ester linkage between D-glucose and galloyl group. Two aromatic protons at  $\delta$  7.14 were attributable to a galloyl group (2H, S, H-2''' and H-6''').

The compound 2 ( $C_{26}H_{30}O_{13}$ ), responding all the tests of flavonoids and glycosides, on acid hydrolysis, furnished sugar in hydrolysate and a aglycone 3. The sugar was identified on Co-PC as D-glucose and the ratio between glucose and aglycone was found to be 1:1 by the  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR data. Permethylation followed by acid hydrolysis, of compound 2, indicated the pyranose form of glucose and the attachment of

aglycone at C-1''' of D-glucose. This was further confirmed by the anomeric proton peak at  $\delta$  5.51. The  $\beta$  glucosidic linkage in glucoside **2** was proved by the enzymatic hydrolysis.

The compound **3** formed a hexa acetate ( $C_{32}H_{32}O_{14}$ ) showing six OH groups. By colour reactions [5, 6] and characteristic UV absorption maxima at 268 and 365 nm, the flavone skeleton was established. A 53 nm bathochromic shift in band I in presence of  $AlCl_3/HCl$  indicated the free 3-OH and 5-OH groups. On addition of NaOMe, appearance of a new band at 325 nm, suggested the presence of 7-OH group. It was further corroborated by a bathochromic shift of 15 nm with NaOAc [7]. Absence of these bands in glycoside suggested the 7-O-glycosidic linkage.

#### **Presence and Position of 2'' - Hydroxy - 3'' - Methyl Butyl Moiety**

The  $^1H$  NMR spectrum of (**3**) was almost similar to that of quercetin with an exception due to signals attributable to a 2'' - hydroxy - 3'' - methyl butyl moiety [ $\delta$  1.63, 3H, d, H-5''; 1.69, 3H, d, H-4''; 3.10, 2H, m, H-1''; 4.23, 1H, m, H-2''; 3.90, m, 1H, H-3'']. The location of hydroxy prenyl substituent at C - 6 of **3** was assigned by comparison of  $^{13}C$  NMR chemical shift of C - 6 and C - 8 (107.3 and 94.3 respectively) with those for quercetin and 6 or 8 substituted 5,7 - dihydroxy flavones. [3, 8, 9, 10]. A signal at  $\delta$  6.70 (1H, s) was due to the proton of position 8 of ring A [11]. This also suggested the trisubstituted nature of ring A and confirmed the position of hydroxy prenyl substituent at C - 6. The presence of a 3', 4' - diOH system was evidenced by a second peak at 267 (sh) nm in the UV spectrum. Signals at  $\delta$  6.80 (1H, d,  $J = 8.5$  Hz, H - 5') 7.40 (1H, dd,  $J = 2.2$  and 8.5 Hz, H - 6') and 7.52 (1H, d,  $J = 2.2$  Hz) are identical to that of disubstituted B ring protons of a quercetin unit. [3]

The spectral data matched with the available literature indicated the compound 3 to be 6 - (2'' - hydroxy - 3'' - methyl butyl) quercetin. The compound 2 was 7 - O -  $\beta$  - D - glucoside of compound 3. Thus finally the structure of 1 could be ascertained as 6 - [2'' - hydroxy - 3'' - methyl butyl] quercetin - 7 - O - (2''' - galloyl) -  $\beta$  - D - glucopyranoside.

## EXPERIMENTAL

The aerial parts of *K. ramosissima* were collected from the adjoining regions and were identified by the Botany Department of this college. A voucher specimen was deposited in the same Department.

### Extraction and Isolation

Air dried and finely powdered aerial parts (3.0 kg.) of *K. ramosissima* were soxhlet extracted with 95% MeOH. The concentrated MeOH extract was dissolved in cold H<sub>2</sub>O and the solution successively partitioned with n hexane, C<sub>6</sub>H<sub>6</sub>, CHCl<sub>3</sub> and EtOAc. The concentrated EtOAc soluble part was chromatographed over a silica gel column (5 x 90 cm.), eluting with CHCl<sub>3</sub>: MeOH with increasing polarity. The CHCl<sub>3</sub>: MeOH (4 : 6) part gave 1.

**Compound (1)** Crystallization with MeOH yielded a yellow coloured microcrystalline powdered compound 1. (Found C: 56.44, H: 4.86, Cal. C: 56.41, H: 4.84) analysed for C<sub>33</sub> H<sub>34</sub> O<sub>17</sub>, M<sup>+</sup> 702, UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm 257, 267 (sh), 368; (+ NaOMe) 293, 362, 452 (dec.); (+ AlCl<sub>3</sub>) 263 (sh), 273, 338, 453; (+ AlCl<sub>3</sub> + HCl) 268, 302 (sh), 365, 422 (dec.); (+ NaOAc) 258, 379, 430 (sh) (dec.). IR  $\nu_{\text{max}}^{\text{KBr}}$  3400, 2915, 1715, 1668, 1648, 1605, 1510, 1380, 1370, 1280, 1220, 1148, 823 cm<sup>-1</sup> EIMS, m/z, 702, [M<sup>+</sup>], 577, [M<sup>+</sup>-125], 550, 388, 370 [388 - 18], 355, 315 [388-73], 314, 301 [388 - 87], 272 [301 - 29], 153,

137, 135, 125.  $^{13}\text{C}$ NMR ; 158.4(s, C - 2), 135.8 (s, C - 3), 178.7(s, C - 4), 162.6 (s, C - 5), 107.5 (s, C - 6), 167.8 (s, C - 7), 94.5 (d, C - 8), 158.6 (s, C - 9), 105.4 (s, C - 10), 122.9 (s, C - 1'), 116.4 (d, C - 2'), 145.8 (s, C - 3'), 148.2 (s, C - 4'), 115.6 (d, C - 5'), 122.9 (d, C - 6'), 30.8 (t, C - 1''), 77.4 (d, C - 2''), 30.1 (d, C - 3''), 25.3 (q, C - 4''), 18.1 (q, C - 5''), 104.5 (d, C - 1'''), 74.1 (d, C - 2'''), 78.3 (d, C - 3'''), 72.3 (d, C - 4'''), 77.2 (d, C - 5'''), 62.5 (t, C - 6'''), 121.1 (s, C - 1'''), 110.7 (d, C - 2''', C - 6'''), 146.5 (s, C - 3''', C - 5'''), 140.1 (s, C - 4''').

**Acetylation of 1** Compound 1 heated with fused sod. acetate and acetic anhydride at  $130^\circ$  for 6 hrs and worked up as usual, yielded an undecaacetate, (Found C : 56.66, H : 4.83 ; cal. C : 56.70, H : 4.81) analysed for  $\text{C}_{55}\text{H}_{56}\text{O}_{28}$ ,  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 60 MHz)  $\delta$  6.70 (s, 1H, H - 8), 7.52 (d, 1H, 2.2 Hz, H - 2'), 7.40 (dd, 1 H, 2.2 and 8.5 Hz., H - 6'), 6.80 (d, 1H, 8.5Hz., H - 5'), 2.40 (s, 3H, 3' - OAc), 2.35 (s, 3H, 4' - OAc), 2.41 (s, 3H, 3 - OAc), 2.47 (s, 3H, 5 - OAc), 3.10 (m, 2H, H - 1''), 4.23 (m, 1H, H - 2''), 3.90 (m, 1H, H - 3''), 1.69 (d, 3H, H - 4''), 1.63 (d, 3H, H - 5''), 2.23 (s, 3H, 3'' - OAc), 4.30 - 4.84 (m, 5H - protons of sugar), 5.51 (d, 1H, 7Hz., Anomeric proton of sugar), 5.10 (dd, 1H, 7.7 Hz C - 2'''), 2.51 (s, 3H, 6''' - OAc), 2.15 (s, 3H, 3''' - OAc), 2.01 (s, 3H, 4''' - OAc), 7.16 (s, 2H galloyl H - 2''', H - 6'''), 2.30 (s, 6H, 3''' and 5''' - OAc), 2.49 (s, 3H, 4''' - OAc).

**Alkaline Hydrolysis of 1.** Compound 1 was dissolved in MeOH and after adding 2% NaOMe, it was kept overnight. The reaction mixture was concentrated under vacuum after neutralizing it with dilute HOAc. An  $\text{Et}_2\text{O}$  soluble part yielded methyl ester of gallic acid. The spectral data of which were found to be identical with the published paper [12].

The  $\text{Et}_2\text{O}$  insoluble part was a yellowish amorphous substance 2,  $\text{C}_{26}\text{H}_{30}\text{O}_{13}$  (found C : 56.77, H : 5.43, Cal. C : 56.73, H : 5.45) EIMS [ $\text{M}^+$ ] 550, UV  $\lambda^{\text{MeOH}}_{\text{max}}$  nm,

258, 270 (sh), 370; (+NaOMe) :290, 365, 455 (dec.) (+AlCl<sub>3</sub>) 263 (sh), 274, 340, 456, (+AlCl<sub>3</sub> +HCl) : 269, 304 (sh), 367, 421 (+NaOAc) 257, 379, 430 (sh) (dec.) ; IR  $\nu_{\text{max}}^{\text{KBr}}$  3405, 2915, 1665, 1646, 1605, 1510, 1382, 1372, 1280, 1220, 1152, 825  $\text{cm}^{-1}$  ; EIMS,  $m/z$ , 550 [ $\text{M}^+$ ], 388, 370, [388 - 18], 355, 315, 314, 301, 272, 153, 137, 135, 125 ;  $^{13}\text{C}$  NMR 158.3 (s, C - 2), 135.4 (s, C - 3), 178.9 (s, C - 4), 162.3 (s, C - 5), 107.7 (s, C - 6), 167.5 (s, C - 7), 94.8 (d, C - 8), 158.9 (s, C - 9), 105.1 (s, C - 10), 123.2 (s, C - 1'), 116.7 (d, C - 2'), 146.1 (s, C - 3'), 148.8 (s, C - 4'), 115.9 (d, C - 5'), 122.6 (d, C - 6'), 30.6 (t, C - 1''), 77.8 (d, C - 2''), 30.0 (d, C - 3''), 25.6 (q, C - 4''), 18.3 (q, C - 5''), 104.8 (d, C - 1'''), 80.1 (d, C - 2'''), 76.9 (d, C - 3'''), 72.6 (d, C - 4'''), 77.5 (d, C - 5'''), 62.1 (t, C - 6''').

Compound 2, yielded an nona acetate,  $\text{C}_{44}\text{H}_{48}\text{O}_{22}$  (found C : 56.86 ; H : 5.19 , cal. C : 56.90 ; H : 5.17),  $^1\text{H}$  NMR (60 MHz,  $\text{CDCl}_3$ )  $\delta$  6.70 (s, 1H, H - 8), 7.52 (d, 1H, 2.2Hz., H - 2'), 7.40 (dd, 1H, 2.2 and 8.5Hz., H - 6'), 6.80 (d, 1H, 8.5Hz., H - 5'), 2.41 (s, 3H, 3' - OAc), 2.37 (s, 3H, 4' - OAc), 2.43 (s, 3H, 3 - OAc), 2.46 (s, 3H, 5 - OAc), 3.12 (m, 2H, H - 1''), 4.22 (m, 1H, H - 2''), 3.92 (m, 1H, H - 3''), 1.69 (d, 3H, H - 4''), 1.63 (d, 3H, H - 5''), 2.26 (s, 3H, H - 3'' - OAc), 4.31 - 4.82 (m, 6H, - protons of sugar), 5.53 (s, 1H, 7Hz., anomeric proton of sugar), 2.50 (s, 3H, 6''' - OAc), 2.13 (s, 3H, 3''' - OAc), 2.08 (s, 3H, 2''' - OAc), 2.03 (s, 3H, 4''' - OAc), 7.16 (s, 2H, galloyl H - 2''', H - 6'''), 2.30 (s, 6H, 3''' and 5''' - OAc), 2.48 (s, 3H, 4''' - OAc).

**Acid Hydrolysis of Compound 2.** Compound 2 was hydrolysed with 7% ethanolic  $\text{H}_2\text{SO}_4$ . After refluxing for about 8 hrs., it yielded aglycone 3 on removal of EtOH. The hydrolysate was neutralised with  $\text{BaCO}_3$  and filtered to remove  $\text{BaSO}_4$ . The filtrate after concentration under vacuum, was examined on PC. (Rf. 0.20) [ n - BuOH - HOAc -  $\text{H}_2\text{O}$

(4 : 1 : 5)] and aniline hydrogen phthalate as spraying reagent to show the presence of D – glucose.

Compound 3. Yellowish microcrystalline powder, (found C : 61.89 ; H : 5.14 ; Cal, C : 61.86 ; H : 5.16), analysed for  $C_{20}H_{20}O_8$ , EIMS [ $M^+$ ] 388 ; UV  $^{MeOH}_{max}$  nm 256, 268 (sh), 300 (sh), 365 ; (+NaOMe) 248 (sh), 325 (dec.), 418 ; (+NaOAc), 258 (sh), 283, 327, 389 (dec.) ; (+AlCl<sub>3</sub>) 274, 305 (sh), 334, 458 ; (+AlCl<sub>3</sub> + HCl) 264, 302 (sh), 360, 418 ; IR  $\nu^{KBr}_{max}$ , 3405, 2915, 1665, 1646, 1605, 1510, 1382, 1372, 1280, 1220, 1152, 825  $cm^{-1}$  ; EIMS, m/z 388 [ $M^+$ ], 370 [ $M^+ - 18$ ], 355, 315, 314, 301, 272, 153, 137, 135, 125 ;  $^{13}C$  NMR 158.6 (s, C – 2), 135.6 (s, C – 3), 179.0 (s, C – 4), 162.5 (s, C – 5), 107.3 (s, C – 6), 163.6 (s, C – 7), 94.3 (d, C – 8), 158.8 (s, C – 9), 105.3 (s, C – 10) ; 123.0 (s, C – 1'), 116.8 (d, C – 2'), 146.3 (s, C – 3'), 148.6 (s, C – 4'), 115.5 (d, C – 5'), 123.0 (d, C – 6'), 30.9 (t, C – 1''), 77.5 (d, C – 2''), 30.1 (d, C – 3''), 25.1 (q, C – 4''), 18.5 (q, C – 5'').

It formed a hexacetate  $C_{32}H_{32}O_{14}$  (found C : 60.08, H : 5.02, Cal. C : 60.0, H : 5.0) ; EIMS ( $M^+$ ) 388 ;  $^1H$  NMR (60 M Hz.,  $CDCl_3$ ) 6.70 (s, 1H, H – 8), 7.52 (d, 1H, 2.2 H<sub>3</sub>) H – 2'), 7.40 (dd, 1H, 2.2 and 8.5 Hz., H – 6'), 6.80 (d, 1H, 8.5 Hz., H – 5'), 2.40 (3, 3H, 3' – OAc), 2.34 (s, 3H, 4' – OAc), 2.41 (s, 3H, 3 – OAc), 2.47 (s, 3H, 5 – OAc), 2.44 (s, 3H, 7 – OAc), 3.10 (m, 2H, H – 1''), 4.23 (m, 1H, H – 2''), 3.90 (m, 1H, H – 3''), 1.69 (d, 3H, H – 4''), 1.63 (d, 3H, H – 5''), 2.24 (s, 3H, 3'' – OAc).

#### Linkage of Aglycone and Gallic Acid with Sugar :

Compound 1 with MeI and Ag<sub>2</sub>O in DMF was taken at room temperature and kept for 2 days and filtered. After drying the filtrate in vacuo, it was hydrolysed with 22% ethanolic H<sub>2</sub>SO<sub>4</sub> for 4 hrs. After usual workup, the methylated sugar was identified by Co

- Pe as 3, 4, 6 - tri - o methyl - D -glucose. Compound 2 was treated in the similar manner, to identify the methylated sugar as 2, 3, 4, 6 - tetra - O - methyl - D - glucose.

#### Enzymatic Hydrolysis :

Compound 2 in MeOH and an equal volume of almond emulsion solution were mixed and left at room temperature for 24 hrs. The hydrolysate after usual workup was examined on PC to show the presence of D - glucose.

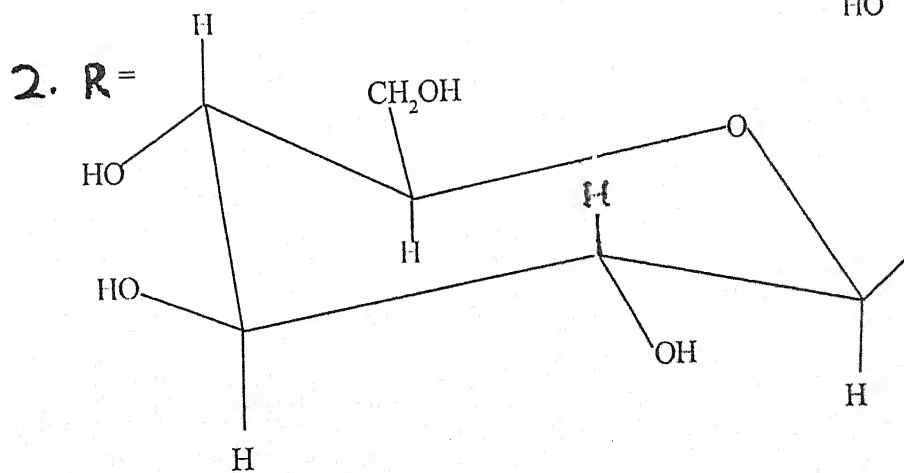
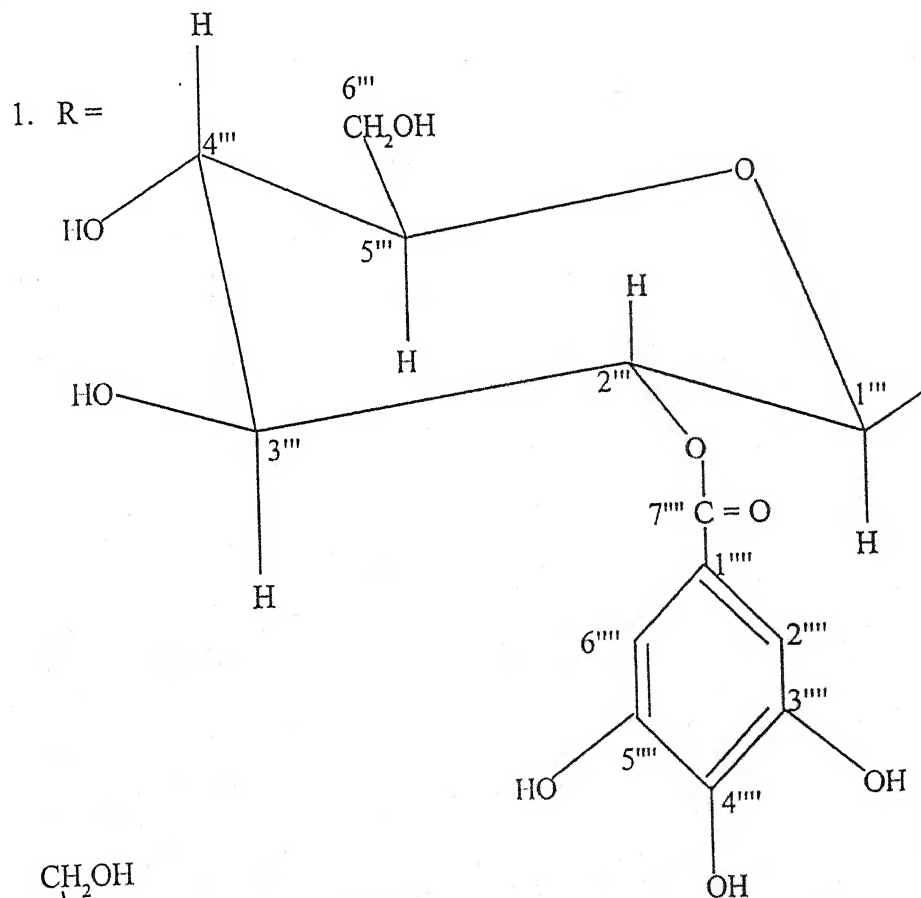
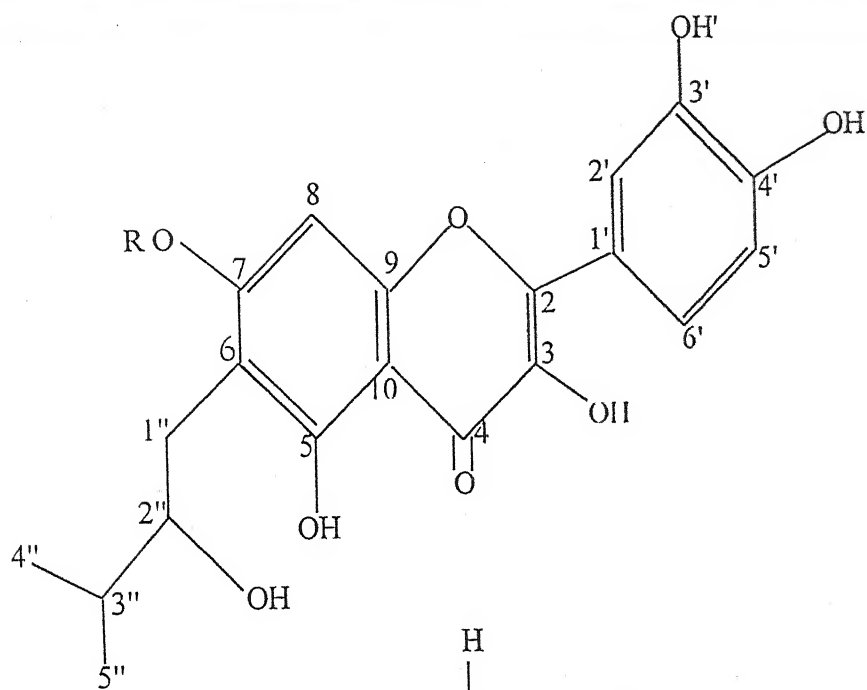
#### ACKNOWLEDGEMENTS

The authors are grateful to Dr. B.K. Bhadoria, Sr. Scientist, I.G.F.R.I., Jhansi for his unforgettable technical support. Our thanks are also due to the Principal B.B. Science College Jhansi for providing lab. facilities and CDRI Lucknow for recording of spectra. We are indebted to late Dr. S.K. Saxena of B.B. Science College Jhansi who identified the plant material.

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## ACCEPTANCE LETTER

Dear Dr. / Mr. Neeraj Srivastava ,

I am pleased to inform you that the abstract of paper entitled  
A.....Triterpeneoid....."3,21-Dihydroxy-olean-12-E-28-Oic  
.....acid".....from.....Celtis.....Coximandelliana..... is accepted for  
ORAL / POSTER presentation in the 19th Conference of the Indian Council of Chemists  
going to be held at Kuvempu University, Shimoga on 27-29th November, 2000.

No TA / DA will be paid by the council to the author/authors of this paper for its  
presentation in the above conference.

Thanking you,

Yours Sincerely



Sectional President

Inorganic / Organic / Physical /  
Anat. & Environment Section

A TRITERPENOID, "3, 21-DIHYDROXY - OLEAN - 12 - EN - 28 - OIC ACID"  
FROM *CELSIA COROMANDELIANA*"

HARIMOHAN GUPTA and NEERAJ SRIVASTAVA

Department of Chemistry, Bipin Bihari Science College, Jhansi, 284001.

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ABSTRACT

*Celsia coromandeliana* Vahl. (Family Scrophulariaceae) is a weed in cultivated grounds that occurs throughout India from Punjab to Ceylon and ascending to 5000 ft. on the Himalayas, extending to Afghanistan, Burma and China. In Sanskrit, the plant is named as *Bhutakeshi* & *Kulahala* and in Hindi, it is called as *Gadartambaku*. The juice of the leaves of this plant is sedative, astringent and is used in diarrhoea and dysentery. The plant juice is used in the skin eruptions and fevers. In this paper, we report the identification of a triterpenoid from the aerial parts of *C. coromandeliana*.

The methanolic extract of the aerial parts of *C. coromandeliana* (Vahl.) yielded a triterpenoid, 3, 21-dihydroxy - olean - 12 - en - 28 - oic acid, molecular formula  $C_{30}H_{48}O_4$ , m.p. 292-293° and  $M^+$  472 (EIMS). It was soluble in methanol and ethanol and responded positively to all the tests of triterpenoids.

It was characterised by the chemical studies and the spectral techniques like IR,  $^1H$  NMR,  $^{13}C$  NMR and mass spectra.

A TRITERPENOID, "3, 21-DIHYDROXY - OLEAN - 12 - EN - 28 - OIC ACID"  
FROM *CELSIA COROMANDELIANA*

HARIMOHAN GUPTA and NEERAJ SRIVASTAVA

Department of Chemistry, Bipin Bihari Science College, Jhansi, 284001.

Key Word Index: *Celsia coromandeliana*, Scrophulariaceae, 3, 21-dihydroxy - olean - 12 - en - 28 - oic acid, triterpenoid.

ABSTRACT:

A triterpenoid 3, 21-dihydroxy - olean - 12 - en - 28 - oic acid was isolated from the methanolic extract of air-dried and powdered aerial parts of *Celsia coromandeliana*. The spectral analysis and chemical studies ascertained the structure of this compound.

.....  
*Celsia coromandeliana* [1]Vahl. (Family Scrophulariaceae) is a weed in cultivated grounds that occurs throughout India from Punjab to Ceylon and ascending to 5000 ft. on the Himalayas, extending to Afghanistan, Burma and China. In Sanskrit, the plant is named as *Bhutakeshi* & *Kulahala* and in Hindi, it is called as *Gadartambaku*. The juice of leaves of this plant is sedative, astringent and is used in diarrhoea and dysentery. The plant juice is used in the skin eruptions and fevers [2]. Due to its interesting medicinal properties, the authors decided to investigate this plant chemically in hope of isolating and identifying some compounds related to these properties.

RESULTS AND DISCUSSION

The compound 1  $C_{30}H_{48}O_4$  was soluble in methanol and ethanol and gave all the tests of triterpenoids [3,4]. The triterpenoidal nature was confirmed by the IR spectrum of the compound 1, which showed peaks at 1396, 1380 and 1320  $cm^{-1}$ .

The triterpenoid was pentacyclic in nature because it gave violet colour with 2,6-di tertiary -butyl-p-cresol in ethanol [5]. In the IR spectrum of the compound 1, a peak at  $1720\text{ cm}^{-1}$  pointed towards the presence of  $-\text{COOH}$  group. On methylation with  $\text{CH}_2\text{N}_2$ , compound 1 yielded methylated ester 2; which also confirmed the presence of free acid group in the compound. IR spectrum ( $3575\text{cm}^{-1}$ ) of the compound 1 was also pointing towards the presence of hydroxyl group(s) in the compound. The number of OH groups were estimated by acetylation of methyl ester 2 with  $\text{Ac}_2\text{O}$  /pyridine, when it formed the acetylated derivative 2a; By the method of Wisenberger [6,7] two hydroxyl groups were established in the molecule. The compound 1 showed the presence of double bond because it gave positive colour test with TNM (tetra nitro methane) [8], which was further confirmed by the IR peaks at  $3032, 1628\text{ cm}^{-1}$ . A peak at  $\nu_{\text{max}}^{\text{KBr}} 2960\text{ cm}^{-1}$  pointed towards the presence of methyl groups, which were found to be seven in number.

The unreactivity of the compound with  $\text{H}_2/\text{Pt}$  at  $280^\circ$  suggested the presence of resistant double bond inside the ring system. The presence of double bond at 12-13 position was inferred by the high terminal absorption at  $185\text{ nm}$  in the UV spectrum, which is a characteristic peak of double bond at this position in oleanane series of majority of triterpenes [9]. This was further confirmed by the upfield NMR peak at  $\delta 5.24$  for the vinylic proton at C -12, a downfield peak at  $\delta 2.15$  for the  $\text{CH}_2$ -11 and another downfield peak at  $\delta 2.40$  for the C-18 proton [10]. In the  $^{13}\text{C}$  NMR spectral data, the signals at  $123.2$  and  $145.1$  were attributable to the C-12 and C-13 confirming the double bond at this position [11]

The  $^1\text{H}$  NMR spectrum of the diacetyl derivative 2a of the methyl ester 2 of the compound 1 indicated the position of  $-\text{OH}$  groups at position 3 ( $\delta 4.03$ , 1 H, dd,  $J=10$  and  $5\text{ Hz}$ , Methenic proton at H-3) and 21 ( $\delta 3.22$ , 1H, dd,  $J = 10.2, 9.0$

Hz, Carbinyllic proton at H-21). Two singlets at  $\delta$  2.01 (3H, C-3, -OAc) and  $\delta$  2.10 (3H, C-21 OAc) indicated the position of -OH groups at C-3 and C-21 and their secondary nature. This was further confirmed by the oxidation of monomethyl ester **2**, with  $\text{CrO}_3$  / pyridine when it gave an oxidation product **2b**. Compound **2b** gave dihydrazone derivative on reaction with 2,4- dinitro phenyl hydrazine, thus, confirming the oxidation product to be diketo derivative and indicated towards the secondary nature of both the -OH groups of the **1**. The Zimmermann test (a specific test for C-3 keto group) [12] of the compound **2b** gave positive results, which further corroborated the position of one keto group at C-3 position. Therefore, OH groups were assigned C-3 and C-21 positions in the compound **1**.

On heating **1** gave a decarboxylated product, **1a**. The  $^1\text{H}$  NMR spectrum of the **1a**, showed a signal at  $\delta$  2.54 (m, 1H) for H-17 proton, while, no such peak for H-17 was observed in the spectra of mono methylated diacetyl derivative **2a**, thus, proving the presence of -COOH group at C-17 position. It was further confirmed by the in the appearance of  $^1\text{H}$  NMR peak at  $\delta$  3.66 (s, 3H) for -COOMe group [9,13] in the **2a**. . In the mass spectra of compound fragment ions at 248 and 203 during Retro - Diels - Alder cleavage further suggested the position 17 of -COOH group [14]. The  $^1\text{H}$  NMR peaks at  $\delta$  0.77, 0.86, 0.93, 0.96, 0.98, 1.02 and 1.08 (each 3H, s) of acetylated derivative were in accordance with the oleanane type ( $\beta$  amyrin) of skeleton [15,16]. Thus, the positions of seven methyl groups were established at  $\text{C}_{23}$ ,  $\text{C}_{24}$ ,  $\text{C}_{25}$ ,  $\text{C}_{26}$ ,  $\text{C}_{27}$ ,  $\text{C}_{29}$  and  $\text{C}_{30}$ .

### EXPERIMENTAL

The aerial parts of *Celsia coromandeliana* were collected from the adjoining regions and were identified by the Botany Department of this college.

Extraction and Isolation 4.0 kg of finely powdered, plant material (aerial parts of *Celsia coromandeliana*) were extracted with methyl alcohol in a flask fitted with reflux condenser. The extracts were diluted with H<sub>2</sub>O and then extracted at room temperature with CHCl<sub>3</sub>, EtOAc and n- BuOH successively. The CHCl<sub>3</sub> extract was defatted with hexane. The hexane insoluble portion was chromatographed on a silica gel column. This column was then run successively with CHCl<sub>3</sub>: CH<sub>3</sub>OH in different proportions. The CHCl<sub>3</sub>: CH<sub>3</sub>OH (97:3) portion showed a single spot on TLC examination using CHCl<sub>3</sub>: CH<sub>3</sub>OH (9:1) solvent system and 10% sulphuric acid as visualising agent. The R<sub>f</sub>. values of fractions (9-16) was found to be same, thus, they were mixed together. After evaporating the solvent, a white-cream amorphous residue was obtained which was examined by TLC. It was purified by crystallising it with methanol when it yielded colourless; needle shaped crystals of the compound 1. The compound 1 showed the entire characteristic reactions of Triterpenoids.

Triterpenoid 1, colourless needle shaped crystals, molecular formula C<sub>30</sub>H<sub>48</sub>O<sub>4</sub> and M<sup>+</sup> 472 (EIMS). (Found C: 76.41, H: 10.28, Cal. C : 76.26, H : 10.17) IR  $\nu$  <sup>KBr</sup><sub>max</sub> 3575, 3032, 1628, 2960, 2895, 2858, 1720, 1634, 1385, 1374, 1396, 1380, 1320, 1370 and 823 cm<sup>-1</sup> EIMS; m/z 472 [M<sup>+</sup>], 471, 455, 440, 410, 248, 207, 203, 190, 189, 175 and 133. <sup>13</sup>C NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$  39.9 (C-1), 28.7 (C-2), 77.4 (C-3), 39.8 (C-4), 56.6 (C-5), 17.9 (C-6), 31.7 (C-7), 38.8, (C-8), 46.5, (C-9), 37.6 (C-10), 24.3, (C-11), 123.2 (C-12), 145. 1(C-13), 42.9 (C-14), 27.6, (C-15), 22.6 (C-16), 47.5 (C-17), 41.7 (C-18), 47.3 (C-19), 31.1 (C-20), 62.4 (C-21), 34.4 (C-22), 27.5 (C-23), 17.1, (C-24), 15.1(C-25), 16.1 (C-26), 25.0 (C-27), 178.4 (C-28), 34.9 (C-29), 22.6 (C-30).

Methylation of 1 The ethereal solution of 300 mg of compound 1 was methylated with an ethereal diazomethane solution when a methyl ester 2 (260mg.) of Triterpenoid 1

was obtained. The compound **2** was analysed for molecular formula  $C_{31}H_{50}O_4$  and  $M^+$  486 (EIMS). [Found C: 76.98, H: 10.37, Cal. C: 76.54, H: 10.28].

The methyl ester **2** (200mg.) was acetylated with  $Ac_2O$ /pyridine, when a acetylated derivative **2a** (165 mg) was obtained. In the acetylated derivative two OH groups were estimated by the method of Wisenberger. The compound **2a** was analysed for the molecular formula,  $C_{35}H_{54}O_6$  and  $M^+$  570 EIMS. [Found C: 73.97, H: 9.55, Cal. C: 73.68, H: 9.48]  $^1H$  NMR spectrum ( $CDCl_3$ , 60 MHz)  $\delta$  0.77 (s, 3 H, Me), 0.86 (s, 3H, Me), 0.93 (s, 3H, Me), 0.96 (s, 3H, Me), 0.98 (s, 3H, Me), 1.02 (s, 3H, Me), 1.08 (s, 3H, Me), 1.22-1.98 (m, 18 H, polymethylene and methyl  $CH_2$  and CH), 2.01 (s, 3H, C-3 OAc), 2.10 (s, 3H, C-21 OAc), 2.15, (t, 2H,  $CH_2$ -11), 2.51 (dd, 1H, 3.6, 9.6 Hz., H-18), 3.22 (dd, 1H, 9.0, 10.2 Hz., Carbinyllic proton at C-21), 3.66 (s, 3H, COOMe at C-17), 4.03 (dd, 1H, 5, 10 Hz., methine proton at C-3), 5.24 (dd, 1H, 6.9, 10.1 Hz., vinylic proton at C-12).

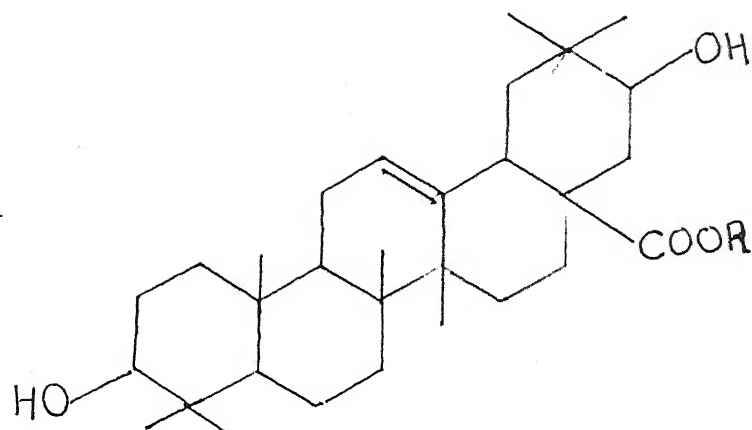
The methyl ester of triterpenoid, **2** (30 mg) was oxidised by taking it with 10 ml of 80% acetic acid and a solution of 200 mg of  $CrO_3$  in 10 ml of pyridine was added to this reaction mixture with constant cooling till a fast orange colour was obtained. The oxidation product (**2b**), obtained after filtration, was dried to get a crystalline mass. It responded positively to the Zimmermann test for keto group presence.

#### ACKNOWLEDGEMENTS

The authors are immensely thankful to Dr. B. K. Bhadoria, Sr. Scientist, I.G.F.R.I. Jhansi for his unforgettable technical support. We are also thankful to the Principal B. B. Science College Jhansi and Director, I.G.F.R.I. for providing lab facilities and CDRI Lucknow for recording of spectra. We are indebted to late Dr. S. K. Saxena of B. B. Science College Jhansi, who identified the plant material.

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1.  $R = H$

2.  $R = -CH_3$



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Post Box No. 46, Bipin Bihari (P.G.) College, Jhansi (U.P.) 284001 INDIA

Dated 28.9.2000

To,  
Dr. H.M. Gupta  
Reader  
Dept. of Chemistry  
B.B. (P.G.) College, Jhansi  
U.P.

Dear Sir,

Thank you very much for your paper entitled "Echinaytic Acid-3-O- $\beta$ -D Galactopyranoside from Ziziphus numularia in collaboration with Mr. Neeraj Srivastava. Hope it would be accommodated in vol. 6 No-1 pr. 2000 of Flora & Fauna.

(Dr. A.K. Srivastava)  
Executive Editor  
Flora & Fauna

# ECHINOCTIC ACID -3-O- $\beta$ -D-GALACTOPYRANOSIDE FROM *ZIZIPHUS*

## NUMMULARIA

HARIMOHAN GUPTA and NEERAJ SRIVASTAVA

Department of Chemistry, Bipin Bihari Science College, Jhansi, 284001.

**Key Word Index:** *Ziziphus nummularia*, Rhamnaceae, echinocystic acid -3-O- $\beta$ -D-galactopyranoside, triterpenoidal saponin glycoside and saponin.

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### Abstract:

The ethanolic extract of finely powdered stems of *Ziziphus nummularia* (Fam. Rhamnaceae) yielded colorless crystals of a triterpenoid saponin 1, identified as "echinocystic acid -3-O- $\beta$ -D-galactopyranoside" by spectral analysis and chemical reactions.

---

### INTRODUCTION

*Ziziphus nummularia* is a thorny small bush or shrub and belongs to the family Rhamnaceae. It is commonly found in the arid and semiarid regions of Indian subcontinent.<sup>1,2</sup> The plant in the vernacular language is known as *Jhadiaber* and is used as a folk medicine in the tropical and semitropical countries. The fruits are cooling and astringent and are used in the bilious affections<sup>2</sup>. The leaves of *Z. nummularia* are applied to scabies and boils<sup>2</sup> and the dried leaves are burnt to inhale the smoke, which is useful in the treatment of coughs and cold.<sup>3</sup> Due to its interesting medicinal properties, the authors decided to investigate this plant chemically in hope of some compounds related to these properties.

## RESULTS AND DISCUSSION

The compound 1  $C_{36}H_{58}O_9$ , gave positive tests for saponins<sup>4</sup>. Appearance of peaks at 1352 and 1345  $cm^{-1}$  in the IR spectrum and the positive tests of triterpenoidal saponins<sup>5,6</sup> were an indication of the presence of triterpenoidal nucleus, which was pentacyclic in nature because it gave violet colour with 2,6-di tertiary -butyl-p-cresol in ethanol<sup>7</sup>. The saponin gave positive colour test with TNM<sup>8</sup> (tetra nitro methane) and showed IR peaks at 3030 and 1625  $cm^{-1}$  to prove the presence of double bond. The IR spectrum of the compound 1 showed the presence of a free acid group, methyl groups and hydroxyl group due to the appearance of peaks at 1725, 2930 and 3410  $cm^{-1}$  respectively. On methylation with  $CH_3N_2$ , saponin 1 yielded a methylated ester 1a, which confirmed the presence of free acid group in the compound. The number of OH groups were estimated by acetylation of methyl ester 1a with  $Ac_2O$  /pyridine, to form the acetylated derivative 1b. By the method of Wisenberger<sup>9,10</sup>, five hydroxyl groups were established in the molecule.

The compound 1, on hydrolysis with 2 N  $H_2SO_4$ , yielded sugar(s) and a sapogenin 2. The sapogenin 2, gave all the colour reactions of triterpenoids<sup>11,12</sup>. The IR spectrum of the sapogenin 2 also suggested the presence of hydroxyl group, double bond, acidic group and methyl groups. The  $^1H$  NMR spectrum of the diacetyl derivative (2b) of the methyl ester (2a) of the sapogenin 2, indicated the position of -OH groups at C-3 and C-16 and their configuration as  $\beta$  and  $\alpha$  respectively due to the peaks at  $\delta$  3.05 (dd,  $J = 10.3, 9.0$  Hz, H-16 $\alpha$ ),  $\delta$  3.88 (dd,  $J=11.4, 4.6$ Hz, H-3  $\beta$ )<sup>13</sup>,  $\delta$  2.20 (3H, C-16  $\alpha$ , -OAc) and  $\delta$  2.08 (3H, C-3  $\beta$ , OAc). C-16 -OH group was assigned  $\alpha$  configuration due to downfield shift of Me-27 proton at  $\delta$  1.25 (s)<sup>13,14</sup> and C-3 -OH was assigned  $\beta$

configuration because of its large coupling constant (dd,  $J = 11.4, 4.6\text{Hz.}$ ).<sup>13,15</sup> The sapogenin **2** showed high terminal absorption at 188 nm, which is a characteristic of 12-13 double bond in most of the triterpenes of oleanane series<sup>16</sup>. A upfield shift<sup>14</sup> at  $\delta$  5.32 for vinyl proton H-12, a downfield shift at  $\delta$  2.21 for H-11 and a peak at  $\delta$  2.18 for H-18 were clearly indicating the presence of double bond at 12-13. In the  $^{13}\text{C}$  NMR spectral data, the signals at 124 and 143.7 were also attributable to the C-12 and C-13<sup>14</sup>. The Seven-methyl groups were shown to be present in the molecule. The  $^1\text{H}$  NMR spectral data [ $\delta$  0.80, 0.84, 0.87, 0.91, 0.93, 1.01 and 1.26 (each 3H, s)] was in accordance with oleanane type skeleton<sup>17,18</sup>, thus the positions of methyl groups were assigned at C-23, C-24, C-25, C-26, C-27, C-29 and C-30. The sapogenin **2** on decarboxylation yielded a decarboxylated sapogenin **2c**; molecular formula  $\text{C}_{29}\text{H}_{48}\text{O}_2$ , m.p.  $194^\circ$ ,  $M^+$  428 (EIMS). The  $^1\text{H}$  NMR spectrum of **2c**, exhibited a signal at  $\delta$  2.63 (m, 1H) for H-17, whereas, no such peak for H-17 was observed in the spectra of mono methylated diacetyl derivative **2b**, thus, corroborating the presence of  $-\text{COOH}$  group at C-17 position. This position was further confirmed by the emergence of a  $^1\text{H}$  NMR peak at  $\delta$  3.60 (s, 3H) for  $-\text{COOMe}$  group<sup>16,19</sup> of its mono methylated diacetyl derivative, **2b**. In the mass spectra of compound fragment ions at 248 and 203 during Retro - Diels - Alder cleavage further suggested the position 17 of  $-\text{COOH}$  group<sup>20</sup>.

The saponin **1** on alkaline hydrolysis did not produce any sugar, thus the possibility of linkage of sugar moiety through  $-\text{COOH}$  group was cancelled<sup>21</sup>. The C-16 position of linkage was also not possible because of highly sterically hindered position of  $\text{C}_{16}\text{-OH}$  group<sup>22</sup>. Therefore, lone possibility of linkage of sugar with sapogenin was through  $-\text{OH}$  at position  $\text{C}_3$ .

The only sugar was found to be D - galactose by the paper chromatography on Whatmann no. 1 filter paper. The presence of D- galactose (Rf. 0.19) as only sugar moiety was confirmed by the Co PC and Co TLC with authentic sample<sup>23</sup> and <sup>13</sup>C NMR spectral data. The D-galactose was present in the pyranose form, which was confirmed by the permethylation<sup>24</sup> followed by the acid hydrolysis of the saponin 1. The presence of 2,3,4,6- tetra -O-methyl -D- galactose (authenticated by the Co PC and Co TLC) in the hydrolysate was a good confirmation of this fact. This also suggested that the C-1 position of the D-galactose be involved in the glycosidic linkage, which was further confirmed by the anomeric proton peak at  $\delta$  4.46. The configuration of glycosidic linkage was established as  $\beta$  from the enzymatic hydrolysis and coupling constant of anomeric proton ( $J = 7.0$  Hz.)<sup>25</sup>.

The above-discussed facts corroborated the structure of the compound as, Echinocystic acid -3-O- $\beta$ -D-galactopyranoside.

### EXPERIMENTAL

The stems of *Ziziphus nummularia* were collected from the adjoining regions and were identified by the Botany Department of this college.

**Extraction and Isolation** The plant material (stems of *Ziziphus nummularia*) were dried, finely powdered then extracted with 80% ethyl alcohol. The extract was concentrated under reduced pressure to get a dark brown viscous mass (850 mg.). This residue was partitioned between Et<sub>2</sub>O and water. The aq. layer was extracted with EtOAc followed by n- BuOH saturated with water. The n- BuOH fraction was subjected to chromatography on a silica gel column eluted with CHCl<sub>3</sub>-MeOH-H<sub>2</sub>O in different ratios giving 46 fractions. Frs 21-36 were rechromatographed on a silica gel column eluted with CHCl<sub>3</sub>-

EtOAc-MeOH (2:1:2) to afford compound **1**. The colourless crystals of compound **1** were obtained on crystallisation with Me<sub>2</sub>CO.

Saponin **1**, colourless crystals, (Found C: 68.36, H: 9.01, Cal. C : 68.14, H : 9.15,) analysed for C<sub>36</sub>H<sub>58</sub>O<sub>9</sub>, M<sup>+</sup> 634; IR  $\nu$ <sup>KBr</sup><sub>max</sub> 3410, 3030, 1625, 2935, 1725, 1630, 1370, 1352, 1345, 1057, 1104, 810 cm<sup>-1</sup> EIMS; m/z 634 [M<sup>+</sup>] 472, 440, 427, 248, 207, 203, 190, 189, 175 and 133. <sup>13</sup>C NMR (300 MHz, DMSO-d<sub>6</sub>)  $\delta$  40.1(C-1), 28.1(C-2), 90.5(C-3), 40.6(C-4), 57.1(C-5), 17.3(C-6), 33.4(C-7), 39.3(C-8), 48.9(C-9), 37.8(C-10), 24.7(C-11), 123.9(C-12), 145.3(C-13), 43.5(C-14), 28.9(C-15), 66.2(C-16), 48.1(C-17), 42.1(C-18), 47.0(C-19), 30.5(C-20), 35.6(C-21), 34.1(C-22), 28.5(C-23), 17.2(C-24), 16.6(C-25), 17.8(C-26), 26.4(C-27), 176.8(C-28), 34.7(C-29), 23.7(C-30), 103.7(C-1'), 74.2(C-2'), 76.8(C-3'), 74.9(C-4'), 76.0(C-5'), 61.7(C-6').

The ethereal solution of 40 mg of compound **1** was methylated with an ethereal diazomethane solution when a methyl ester (**1a**) (30 mg.) of saponin **1** was obtained. Methyl ester of saponin **1a** analysed for molecular formula, C<sub>37</sub>H<sub>60</sub>O<sub>9</sub> and M<sup>+</sup> 648 (EIMS). [Found C: 68.18 %, H: 9.35 %, Cal. C: 68.52 %, H: 9.26 %]

The methyl ester **1a** (25 mg.) was acetylated with Ac<sub>2</sub>O/pyridine, when an acetylated derivative **1b** (18 mg) was obtained. The compound **1b** analysed for the molecular formula, C<sub>47</sub>H<sub>70</sub>O<sub>14</sub> and M<sup>+</sup> 859 EIMS. [Found C: 65.5, H: 8.29, Cal. C: 65.73, H: 8.16] <sup>1</sup>H NMR spectrum (CDCl<sub>3</sub>, 60 MHz)  $\delta$  0.98 (s, 3H, C-23), 0.81 (s, 3H, C-24), 0.88 (s, 3H, C-25), 0.87 (s, 3H, C-26), 1.12 (s, 3H, C-27), 0.86 (s, 3H, C-29), 0.95 (s, 3H, C-30), 1.15-2.05 (m, 18H, polymethylene and methyl CH<sub>2</sub> and CH), 5.25 (dd, 1H, 4.9 Hz, 12-13 double bond), 3.12 (dd, 1H, 8.14, 8.0 Hz, C-3 H), 3.04 (dd, 1H, 10.25, 9.10 Hz, C16-1H), 3.22 (s, 3H, -COOCH<sub>3</sub>), 2.01 (s, 3H, 16-OAc), 2.19 (d, 1H, 4.2 Hz, 18 $\beta$ -1H), 2.16 (dd, 2H, 4.3 Hz,

CH<sub>2</sub>-11), 3.66-4.26(m, 6H, Protons of sugar moiety), 4.46 (d, 1H, 7 Hz, 1' anomeric proton), 2.02(s, 3H, 2' OAc), 2.01(s, 3H, 3'OAc) 1.94(s, 3H, 4'OAc) and 1.98 (s, 3H, 6' OAc).

The compound **1** (900 mg.) was hydrolysed by heating it with 20 ml. of ethanolic 2N sulphuric acid for about 5 hours on a water bath. The solution was extracted with ether and ethereal layer yielded sapogenin **2**, which was recrystallised from methanol to give colourless needles. The aq. layer after usual workup was examined on PC (Rf. 0.19) using n-BuOH-HOAc – water (4: 1: 5) as solvent system and aniline hydrogen phthalate as spraying agent, to show the presence of D-galactose.

Sapogenin **2**, colourless needles, was studied for molecular formula C<sub>30</sub>H<sub>48</sub>O<sub>4</sub>, and M<sup>+</sup> 472 ( EIMS ). It responded all the reactions of triterpenoids and was soluble in Ethanol and feebly soluble in ethyl acetate. [Found C: 75.84, H: 10.09; Cal. C: 76.23, H: 10.14] IR  $\nu^{\text{KBr max cm}^{-1}}$ : 3402(OH group), 3018, 1622 (double bond), 2855, 1373(methyl group), 1710 (COOH group) and 1405, 1380, 1312 (Triterpenoidal nucleus). EIMS m/z 472 [M<sup>+</sup>], 440, 427, 248, 207, 203, 190, 189, 175 and 133. <sup>13</sup>C NMR (300 MHz, DMSO-d<sub>6</sub>) 39.5 (C-1), 28.6 (C-2), 90.4 (C-3), 39.4 (C-4), 57.8 (C-5), 17.1 (C-6), 33.2 (C-7), 40.7 (C-8), 48.4 (C-9), 38.1 (C-10), 25.4 (C-11), 124.0 (C-12), 143.7(C-13), 42.2 (C-14), 28.9 (C-15), 67.6 (C-16), 46.8 (C-17), 42.1 (C-18), 47.1 (C-19), 32.0 (C-20), 35.1 (C-21), 32.8 (C-22), 28.6 (C-23), 18.3 (C-24), 15.4 (C-25), 17.0 (C-26), 26.0 (C-27), 178.6 (C-28), 34.1 (C-29), 23.6 (C-30).

Sapogenin **2** (350 mg.) was methylated with diazomethane to yield methyl ester derivative **2a** as crystals (310 mg.) from methanol, which was analysed for mol. for. C<sub>35</sub>H<sub>54</sub>O<sub>6</sub> and M<sup>+</sup> 570 EIMS.

The methyl ester **2a** (225 mg.) was acetylated with acetic anhydride to get diacetylated methyl ester derivative (**2b**) of sapogenin **2** as colourless crystals (190 mg.) from acetone. Diacetylated Derivative **2b** was analysed for molecular formula  $C_{35}H_{54}O_6$  and  $M^+$  570 EIMS [found: C: 74.10%, H: 9.57; Cal. C: 73.38, H: 9.47].  $^1H$  NMR spectrum ( $CDCl_3$ , 60 MHz)  $\delta$  0.97 (s, 3H, C-23 Me group), 0.74(s, 3H, C-24 Me group), 0.91(s, 3H, C-25 Me group), 0.89 (s, 3H, C-26 Me group), 1.26 (s, 3H, C-27 Me group), 0.84 (s, 3H, C-29 Me group) 0.96 (s, 3H, C-30 Me group), 1.30-2.03 (m, 18H, Polymethylene and methyl  $CH_2$  and CH), 2.08 (s, 3H, (C-3-OAc), 2.18 (s, 1H, H-18), 2.20 (s, 3H, C-16-OAc), 2.21 (s, H-11), 3.05 (dd, 1H, 10.3, 9.0 Hz., H-16 $\alpha$ ), 3.60 (s, 3H, -COOMe).

**Permethylation and Hydrolysis of 1** The saponin **1** was subjected to the permethylation by taking it (20 mg.) with MeI (1.0 ml.) and silver iodide (16 mg.) in dimethyl formamide for 48 hours at room temperature and filtered. The solution was then hydrolysed by using Killiani's mixture (HCl: AcOH: Water 5: 12: 18). . On addition of excess of water to the mixture, the methylated sapogenin was precipitated out. The aq. part on usual workup gave the methylated sugar, which was identified as 2,3,4,6 tetra - O -methyl - D-galactose by TLC (toluene: methyl alcohol 4:1). (Confirmed by Co-PC and Co-TLC).

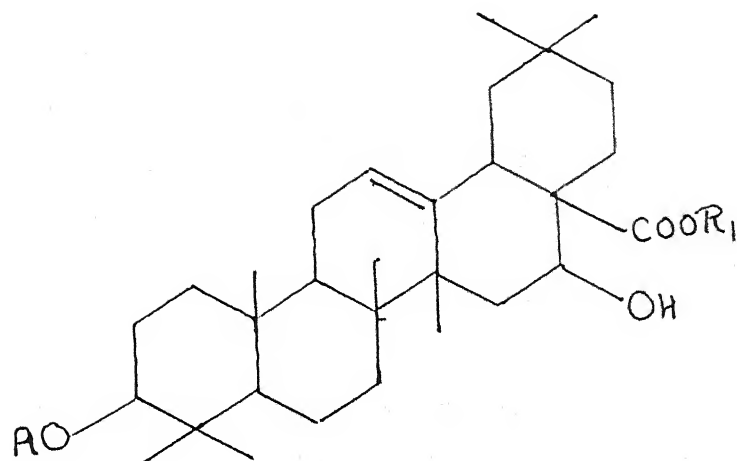
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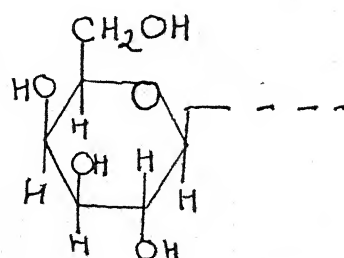
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1.  $R =$



$R_1 = H$

1a.  $R = \text{Gal.}$

$R_1 = -CH_3$

2.  $R = H$

$R_1 = H$

2a.  $R = H$

$R_1 = -CH_3$

## ALLELOCHEMICALS OF SOME PLANTS OF FAMILY SCROPHULARIACEAE

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### ABSTRACT

The secondary metabolites (allelochemicals) Alkaloids, viz. Flavonoids, Iridoids, Isoflavonoids, Saponins, Sterols etc. present in various members of family Scrophulariaceae are reviewed.

Tables : 00

Figures : 00

References : 22

KEY WORDS : Allelochemicals, Flavonoids, Iridoids, Alkaloids, Isoflavonoids, Sterols, Saponins.

The Scroph plants (Scrophulariaceae) are found in ranges, grazing lands and cultivated fields and consist of 220 genera and 3,000 species. These plants are rich in secondary metabolites (allelochemicals) which often directly or indirectly affect human/ animal health when ingested either due to therapeutic value or toxicity.

The flavonoids, most wide spread group of the naturally occurring plant constituents are important and of interest due to their numerous physical and biological activities. Flavones are commonly found in family Scrophulariaceae. Its various genera viz, *Veronica agretis*, *V. opaca*, *V. ceratocarpa*, *V. persica*, *V. filiformis*, *V. autria*, *V. hederacarpa*, *Grateola rateola*, *G. officinalis*, *G. linifolia*, *Linaria arvensis* and *Sopubia delphinifolia* [6-8, 13-15] have been investigated, for their flavonoidal constituents.

Russian scientists [20] have identified acetylpectolinarin, pectolinarin, pectolinarigenin, linaroside and three acacetin derivatives in *Linaria kurdica*. Flavone aglycone acacetin was one of the acacetin derivatives, eluted with  $\text{CHCl}_3$ . The 2nd flavone glucoside eluted with 5% MeOH in  $\text{CHCl}_3$  was identified as acacetin - 7-O-B-D glucopyranoside. Elution of polyamide columns with 50% MeOH in  $\text{CHCl}_3$  yielded the 3rd component which was identified as acacetin - 7-O rutinoside (Linarin).

Apart from pectolinarigenin found in *L. kurdica*, its 7-O-rutinoside, 7-O-robinoside and 4'''-acetyl derivative were isolated from the powdered aerial parts of *Kickxia aegyptiaca* [12]. Pectolinarin, found in *L. kurdica* and its acetyl derivatives were

found for the first time in *L. dalmatica* and *Linaria* spp. [11]. Pectolinarigenin and salvigenin alongwith a new flavone glycoside cirsimaritin 5-galactoside were isolated from *Striga aspera* [3]. Extraction of whole dried plant of *K. lanigera* with methanol followed by T.L.C., afforded two flavones which were identified as 5,6,7-trimethoxy-flavone and 4,5,6,7-tetramethoxy flavone (tetra- o - methylscutellarein) [17].

During recent years a new class of naturally occurring compounds known as methylcyclopentanoid monoterpenes or iridoids has gained increased recognition as they provide a structural link between terpenes and alkaloids.

Eleven iridoids, eight of which were new compounds and five flavonoids were isolated from six *Linaria* species (*L. dalmatica*, *L. genistifolia*, *L. pelisseriana*, *L. simplex*, *Linaria* spp. and *L. vulgaris*). Main iridoid glucoside antirrhinoside was found for the first time in five species, while, linaroside and 5-O-glucosylantirrhinoside in all the six spp. The structures of six new iridoids were determined [11]. Three iridoid esters of an iridoid glucoside named iridolinarins A, B, and C were isolated from whole plant of *L. japonica* [16].

One of the isomeric forms of flavones is isoflavonoid which is often referred as phytoestrogen because it increases milk, meat production in animals and also produces "Infertility syndrome". Although most of the isoflavonoids were isolated from family Leguminosae yet, reports are available from family Scrophulariaceae too. Recently two new isoflavonoids [19,20] 5,7-dihydroxy, 6-prenyl,

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Oct. 10th , 2000

To,

Editor,  
Indian Journal of Chemistry,  
Sec B. National Institute of Science Communication  
Dr. K.S. Krishnan Marg,  
New Delhi. -110012

Dear, Sir,

We have isolated a flavonoid glycoside from a local plant *Kickxia ramosissima* ( Fam. Scrophulariaceae ), on which a paper titled " Structure of a Novel Flavonoid Glycoside From *Kickxia ramosissima* " has been written.

We are sending three copies of the manuscript of the aforesaid paper attached with this letter for publishing it in the coveted journal of " Indian Journal of Chemistry " Sir, kindly consider this paper if you find it compatible to be published in your journal.

Thanking you,

Yours sincerely,

( H.M. Gupta )

# STRUCTURE OF A NOVEL FLAVONOID GLYCOSIDE FROM *KICKXIA*

## *RAMOSISSIMA*

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The ethyl acetate soluble fractions of aerial parts of *Kickxia ramosissima* (Wall.) Janchen Syn. *Linaria ramosissima* Wall. (Scrophulariaceae) yielded a microcrystalline compound identified as "8-prenyl-chrysoeriol-4'-O- $\beta$ -D-xylopyranosyl- (1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl- (1 $\rightarrow$ 6)- $\beta$ -D-galactopyranoside" by spectral analysis and chemical reactions.

*Kickxia ramosissima* (Wall.) Janchen syn. *Linaria ramosissima* belongs to family Scrophulariaceae and occurs throughout India usually on rocky and stony places, hanging downwards from the cervices, and fissures of walls of old buildings. The plant is vernacularly known as *Bhintgalodi* or *Kanodi* and is useful in the treatment of diabetes<sup>1</sup>. The authors, herein, report the presence of a novel flavonoid glycoside, 8-prenyl-chrysoeriol-4'-O- $\beta$ -D-xylopyranosyl- (1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl- (1 $\rightarrow$ 6)- $\beta$ -D-galactopyranoside, identified on the basis of spectral and chemical evidences.

Compound 1 (C<sub>37</sub>H<sub>46</sub>O<sub>19</sub>) obtained as a light yellowish crystalline substance, showed +ve Shinoda test<sup>2</sup> and Molisch's test for flavonoids and sugars respectively indicating flavonoidal and glycosidic nature of compound. The UV  $\lambda_{\text{max}}^{\text{MeOH}}$  268 and 342 nm indicated a flavonoidal skeleton. The IR peak at 3425 cm<sup>-1</sup> pointed towards the presence of OH group(s). The peracetylation of compound 1 (NaOAc + Ac<sub>2</sub>O) yielded a derivative which was found to be decaacetate by the Wisenberger method, due to 10 OH groups. The appearance of chemical shifts at  $\delta$  2.02 – 2.47 integrating for 30 protons, confirmed the presence of 10 OH groups in the molecule.

The compound 1, on acid hydrolysis, furnished sugar in hydrolysate and an aglycone 2. The sugars were identified on Co-PC as D-galactose, L-arabinose and D-xylose and the ratio between aglycone and three sugars was found to be 1:1:1:1 by the  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR data. By colour reactions<sup>3,4</sup> and characteristic UV absorption maxima at 269 and 346 nm, the flavone skeleton was established in the compound 2. In the IR spectrum of the 2, two peaks at  $\nu_{\text{max}}^{\text{KBr}}$  3428  $\text{cm}^{-1}$  and 2825  $\text{cm}^{-1}$ , suggested the presence of hydroxyl and methoxyl groups in the compound. By the Zeisel's method, one methoxyl group was determined in the compound. The UV and  $^1\text{H}$  NMR spectrum of the 2 was almost similar to that of the chrysoeriol<sup>5</sup>, thus, the position of the methoxyl group was assigned at C-3'. A sharp singlet at  $\delta$  3.88 integrating for 3 protons was a confirmation of the presence of the one  $-\text{OCH}_3$  group at position 3' <sup>6</sup>. Compound 2 formed a triacetate  $\text{C}_{27}\text{H}_{26}\text{O}_9$  showing three OH groups. On adding NaOMe a stable bathochromic shift of 56 nm without decrease in intensity, was an evidence of the presence of a  $-\text{OH}$  group at C-4' position in the ring B<sup>7</sup>. This shift was absent in glycoside, thus, pointing towards the 4'-O glycosidic linkage. A bathochromic shift of 42 nm in band I in presence of  $\text{AlCl}_3/\text{HCl}$  was due to the presence of a free 5-OH group in the flayonoid<sup>7</sup>. On adding NaOAc, a bathochromic shift of 28 nm in the band II suggested the presence of a  $-\text{OH}$  group at C-7, which was further corroborated by the appearance of a new band at 328 nm on adding NaOMe<sup>8</sup>.

The  $^1\text{H}$  NMR spectrum of 2 was almost similar to that of chrysoeriol with an exception due to signals attributable to a 3, 3-dimethyl allyl group [ $\delta$  3.42, d, 2H, 6.3 Hz. H-1''; 5.21, t, 1H,  $J = 6.0$  Hz., H-2''; 1.66, s, 3H, H-4'' and 1.79, s, 3H, H-5'']<sup>9,10</sup>. The location of prenyl unit at C-8 was undoubtedly determined by the comparison of  $^{13}\text{C}$  NMR chemical shifts of C-6 and C-8 [97.1(d) & 105.3 (s)]

respectively] of the 6 or 8 substituted 5, 7- dihydroxy flavones<sup>11</sup>. A signal at  $\delta$  6. 48(1 H), assigned to H-6 of the ring A<sup>12</sup>. This proved the tri substituted nature of the ring A and confirmed the attachment of 3, 3- dimethyl allyl unit at C-8.

The compound FG 1 was hydrolysed with Killiani's mixture<sup>13</sup> at room temperature, which liberated first D-xylose, followed by L- arabinose and then D- galactose, therefore, D-xylose was terminal sugar and D-galactose was linked to the aglycone. On column chromatography of contents over Si gel, using methanol as eluant, a mixture of three proaglycones was obtained, which were assigned as 1a, 1b and 1c.

The proaglycone 1a was hydrolysed with 7% sulphuric acid, when it yielded aglycone and D-galactose (confirmed by Co-PC and Co TLC). 1a was hydrolysed by enzyme emulsin, when it gave D-galactose and aglycone, therefore, the D-galactose was linked to the aglycone by the  $\beta$  linkage. The proaglycone 1a was permethylated by the method of Khun<sup>14</sup>, followed by hydrolysis, which gave 2, 3, 4, 6-tetra-O-methyl -D-galactose (identified by Co-PC and Co-TLC), thus, showing that C-1 of the D- galactose was linked to the 4' of the aglycone. It also suggested that D-galactose was present in the pyranose form.

The 1b on acid hydrolysis with 7% sulphuric acid, yielded aglycone, D-galactose and L-arabinose (confirmed by the Co PC and Co TLC). The 1b was also permethylated by the Khun's procedure and then hydrolysis to yield 2, 3, 4-tri-O-methyl-D-galactose and 2, 3, 4-tri-O-methyl-L-arabinose (identified by Co-PC and Co-TLC). This suggested that L- arabinose was linked to the D-galactose by (1 $\rightarrow$  6) linkage. It was also an indication of the fact that both the sugars were present in the pyranose form.

1c was also hydrolysed in the same manner to yield the aglycone FG 2 (confirmed by the mmp, Co-TLC and Co-PC) and sugars, D-galactose, L-arabinose and D-xylose (confirmed by the Co-PC and Co-TLC). On permethylation, followed by the hydrolysis compound 1c yielded 2, 3, 4-tri-O-methyl -D-galactose, 3,4-di-O-methyl-L-arabinose and 2, 3, 4-tri-O-methyl-D-xylose. Thus, clearly pointing that D-xylose was linked to L-arabinose via. (1→2) linkage and that all the sugars were present in the pyranose form.

The glycoside 1 was first hydrolysed by enzyme almond emulsin<sup>15</sup>, when it yielded aglycone FG 2, terminal sugar D-xylose and a disaccharide unit. This was a clear indication of  $\beta$  glycosidic linkage between D-xylose and disaccharide unit and also between aglycone and disaccharide. The hydrolysed product having disaccharide unit was further hydrolysed by the enzyme takadiastase. It liberated the D-galactose and L-arabinose, thus, suggesting the presence of a  $\alpha$  linkage between L-arabinose and D-galactose. The spectral data matched with the available literature indicated the compound 2 to be 8-prenyl-chrysoeriol. The compound 1 was 8-prenyl-chrysoeriol-4'-O- $\beta$ -D-xylopyranosyl-(1→2)- $\alpha$ -L-arabinopyranosyl-(1→6)- $\beta$ -D-galactopyranoside.

## EXPERIMENTAL

The aerial parts of *K. ramosissima* were collected from the adjoining regions and were identified by the Botany Department of this college. A voucher specimen was deposited in the same Department.

Air-dried and finely powdered aerial parts (3.0 kg.) of *K. ramosissima* were soxhlet extracted with 95% MeOH. The concentrated MeOH extract was dissolved in cold H<sub>2</sub>O and the solution successively partitioned with n

hexane,  $C_6H_6$ ,  $CHCl_3$  and EtOAc. The concentrated EtOAc soluble part was chromatographed over a silica gel column (5 x 90 cm.), eluting with  $CHCl_3$ : MeOH with increasing polarity. The  $CHCl_3$ : MeOH (1: 9) part gave 1.

Compound 1 was obtained on crystallisation with methanol as yellowish micro-crystalline compound. [Found C: 55.34; H: 6.16; Cal. C: 55.92; H: 5.79] analysed for Molecular formula  $C_{37}H_{46}O_{19}$  and  $[M^+]$  794[EIMS]. UV  $\lambda_{max}^{MeOH}$  nm 243, 246 (sh), 268, 290 (sh) and 342; (+NaOMe) 270, 320 and 365; (+ $AlCl_3$ ) 260, 278, 294, 361 and 386; (+ $AlCl_3$ +HCl) 258, 278, 291(sh), 346 and 382(sh); (+NaOAc) 272, 319 and 359 and (+NaOAc+ $H_3BO_3$ ) 268 and 340. IR  $\nu_{max}^{KBr}$  3425, 2985, 2820, 1670, 1645, 1600, 1505, 1385, 1368, 1285, 1218, 1145, 828  $cm^{-1}$ . EIMS, 794  $[M^+]$  and  $m/z$  = 661, 529, 368, 367, 353, 340, 313, 312, 299, 165, 164, 148, 137 and 136.  $^{13}C$ NMR; 164.6 (s, C-2), 103.9 (s, C-3), 182.2 (s, C-4), 159.3, (s, C-5), 97.8(d, C-6), 157.7(s, C-7), 104 (s, C-8) 157.2 (s, C-9), 104.9 (s, C-10), 124.9 (s, C-1'), 111.7 (d, C-2'), 150.6 (s, C-3'), 151.4 (s, C-4'), 115.2, (d, C-5'), 120.7(d, C-6'), 61.1(s, C-3'- $OCH_3$ ), 22.8 (t, C-1''), 124.1 (d, C-2''), 131.0 (d, C-3''), 25.4(q, C-4), 17.8(q, C-5''), 103, 2 (d, C-1'''), 73.8 (d, C-2'''), 76.4 (d, C-3'''), 75.3 (d, C-4'''), 76.5 (d, C-5'''), 70.1 (t, C-6'''), 101.6 (d, C-1'''), 79.7 (d, C-2'''), 73.0 (d, C-3'''), 68.1 (d, C-4'''), 63.3 (t, C-5'''), 104.9 (d, C-1'''), 74.6 (d, C-2'''), 76.8 (d, C-3'''), 70.2 (d, C-4'''), 66.1 (t, C-5''').

Compound 1 was heated with fused sod. acetate and acetic anhydride at  $130^\circ$  for 6 hrs and worked up as usual, when it yielded decaacetate, [found C: 56.36; H: 5.59; Cal. 56.34; H: 5.44 analysed for molecular for.  $C_{57}H_{66}O_{29}$ ,  $M^+$  1214,  $^1H$  NMR ( $CDCl_3$ , 90 MHz)  $\delta$  6.23 (1H, s, H-3), 6.46 (1H, s, H-3), 7.85 (1H, d,  $J=1.5$  Hz., H-2'), 7.03 (1H, d, 8.5 Hz., H-5'), 7.51 (1H, dd, 2.2 & 8.5 Hz., H-6'), 3.39 2H, d, 6.3 Hz., H-1''), 5.24

(1H, t, 6.0 Hz., H-2"), 1.69 (3H, s, H-4"), 1.82 (3H, s, H-5"), 3.81 (3H, s, 3' -OCH<sub>3</sub>), 2.47 (3H, s, 5- OAc), 2.40 (3H, s, 7-OAc), 4.74 (1H, d, 7.2 Hz., 1''' anomeric proton), 4.85 (1H, d, 5.2 Hz., 1'''' anomeric proton), 4.92 (1H, d, 7.2 Hz., 1''''' anomeric proton), 3.28-4.68 (16H, m, protons of sugar), 2.07 (3H, s, 2''' -OAc), 2.11 (6H, s, 3''' & 4''' -OAc), 2.09 (6H, s, 2'''' -OAc & 3'''' -OAc), 2.04 (6H, s, 2''''' & 4''''' -OAc), 2.02 (3H, s, 3''''' -OAc).

Compound 1 was hydrolysed with 7% ethanolic H<sub>2</sub>SO<sub>4</sub>. After refluxing for about 8 hrs., it yielded aglycone 2 on removal of EtOH. The hydrolysate obtained from the acid hydrolysis, was neutralised with BaCO<sub>3</sub> and filtered to remove BaSO<sub>4</sub>. The filtrate after concentration under vacuum was examined on PC. (Rf. 0.20) [ n - BuOH - HOAc - H<sub>2</sub>O (4 : 1 : 5)] and aniline hydrogen phthalate as spraying reagent to show the presence of D - xylose, L- arabinose and D- galactose.

Compound 2 was a yellowish microcrystalline powder, [found C: 68.24; H: 5.81; Cal. C: 68.48 and H: 5.43] and analysed for molecular formula C<sub>21</sub>H<sub>20</sub>O<sub>6</sub>, M<sup>+</sup> 368 (EIMS). UV <sup>MeOH</sup><sub>max</sub> nm 241, 251(sh), 269 and 346; (+NaOMe) 264, 278 (sh), 328 (sh) and 402; (+AlCl<sub>3</sub>) 263, 274, 295, 364 (sh), and 391; (AlCl<sub>3</sub>+HCl) 258, 274, 293, 352 and 388; (+NaOAc) 269, 322 and 394; (+NaOAc+H<sub>3</sub>BO<sub>3</sub>) 266 and 348; IR  $\nu$ <sup>KBr</sup><sub>max</sub> 3428, 2980, 2825, 1668, 1642, 1610, 1500, 1386, 1374, 1282, 1226, 1149 and 821. EIMS, m/z, 368 [M<sup>+</sup>] 353, 340, 313, 312, 299, 165, 164, 148, 137 and 136; <sup>13</sup>C NMR,  $\delta$  164.9 (s, C-2), 103.7 (d, C-3), 182.6 (s, C-4), 159.7 (s, C-5), 97.1(d, C-6), 158.4 (s, C-7), 105.3 (s, C-8), 156.8 (s, C-9), 104.6 (s, C-10), 121.2 (s, C-1'), 112.4 (d, C-2'), 148.4 (s, C-3'), 153.3 (s, C-4'), 112.9 (d, C-5'), 119.8 (d, C-6'), 61.7 (q, -OCH<sub>3</sub>), 22.3 (t, C-1''), 123.6 (d, C-2''), 131.4 (s, C-3''), 25.9 (q, C-4'') and 17.1 (q, C-5'').

On acetylation it formed a triacetate C<sub>27</sub>H<sub>26</sub>O<sub>9</sub>, M<sup>+</sup> 494 [found C : 66.09; H: 5.33; Cal.

C: 65.59, H: 5.26], EIMS ( $M^+$ ) 494;  $^1\text{H}$  NMR (90 MHz,  $\text{CDCl}_3$ );  $\delta$  6.26 (1H, s, H-3), 6.48 (1H, s, H-6), 7.82 (1H, d, 1.5 Hz., H-2'), 6.82 (1H, d, 8.5 Hz., H-5'), 7.49 (1H, dd, 2.2 & 8.5 Hz, H-6'), 3.42 (2H, d, 6.3 Hz., H-1''), 5.21 (1H, t, 6.0 Hz., H-2''), 1.66 (3H, s, H-4''), 1.79 (3H, s, H-5''), 3.88 (3H, s, 3'-OCH<sub>3</sub>), 2.49 (3H, s, 5-OAc), 2.42 (3H, s, 7-OAc) and 2.32 (3H, s, 4'-OAc).

**Hydrolysis of 1 with Killiani's mixture.** The flavonoid glycoside FG 1 (400 mg.) and Killiani's mixture (70 ml. HCl: Acetic acid: Water, 15: 35: 50) were taken at room temperature and left for 4 days. The reaction mixture was extracted with n- butanol. Butanol extract on TLC (BAW) examination showed the presence of three compounds. This extract on concentration was subjected to the column chromatography over a column of silica gel and eluting the column with chloroform: methanol in different proportions, to separate the three proaglycones.

**Proaglycone 1a** It analysed for the molecular for.  $\text{C}_{27}\text{H}_{30}\text{O}_{11}$  and  $M^+$  530. (found C = 61.36, H= 5.78 Cal. C= 61.13, H= 5.67) The 1a (50 mg.) was permethylated by taking it with methyl iodide (5.0 ml.) and silver oxide in dimethyl formamide (8.0 ml.) and kept for two days at room temperature. The reaction mixture was filtered and residue was washed with dimethyl formamide. The filtrate was concentrated under reduced pressure to a syrupy mass, which was hydrolysed with 22% sulphuric acid, to give aglycone and methylated sugar. After separation of the aglycone, the aqueous part gave 2, 3, 4, 6-tetra-O-methyl-D-galactoside, which was identified by Co-PC and Co-TLC (butanol: acetic acid: water (4:1:5) and aniline hydrogen phthalate as spraying reagent).

**Proaglycone 1b**, the proaglycone 1b was permethylated and hydrolysed, when it exhibited the presence of aglycone (identified by m.m.p., Co-TLC and Co-PC with

authentic sample). The aq. hydrolysate revealed the presence of two methylated sugars identified as 2, 3, 4-tri-O-methyl-D-galactose and 2, 3, 4-O-methyl-L-arabinose (identified by Co-PC and Co-TLC).

**Proaglycone 1c** It analysed for the molecular formula  $C_{37}H_{46}O_{19}$  and molecular weight 794 (EIMS). [C= 55.34, H= 6.16, Cal. C= 55.92, H= 5.79]. It was permethylated and hydrolysed to get the aglycone (identified by m.m.p., Co TLC and Co-PC) after filtration. The aq. hydrolysate showed the presence of three sugars, 2, 3, 4-tri-O-methyl-D-galactose, 3, 4-di- O-methyl-L-arabinose and 2, 3, 4-tri-O-methyl-D-xylose.

**Enzymatic Hydrolysis** The solution of compound 1 (50 mg.) in ethanol (30 ml.) was suspended in an almond emulsin solution (30 ml.) and the mixture was kept for three days at room temperature. When it clearly revealed the presence of aglycone, D-xylose and a disaccharide sugar unit. Then the takadiastase enzyme was added in the reaction mixture and was again kept for three days. The ppt. was separated by filtration and hydrolysate was subjected to PC with authentic sample, when it showed three spots and revealed clearly the presence of D-galactose, D-xylose and L-arabinose (confirmed by Co-PC and Co-TLC with authentic sample).

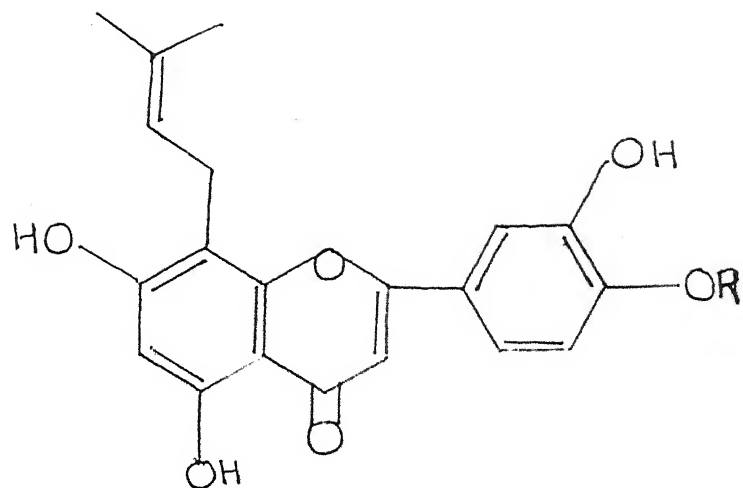
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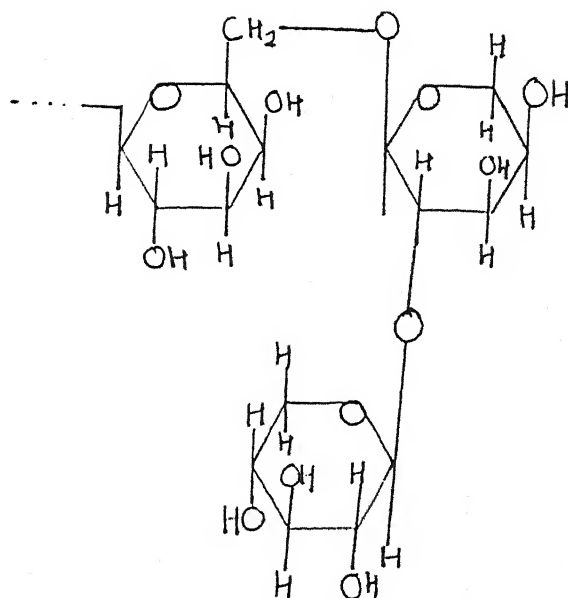
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1 R =



2. R = H